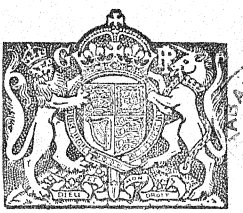
Memoirs of the Department of Agriculture in India

BOTANICAL SERIES

Vol. XVII



IMPERIAL INSTITUTE OF AGRICULTURAL RESEARCH, PUSA

Calcutta: Government of India
Central Publication Branch
1931

Price 1 anna or 1½d.

EDITED BY

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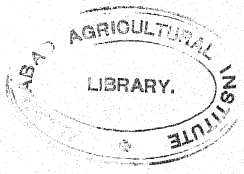

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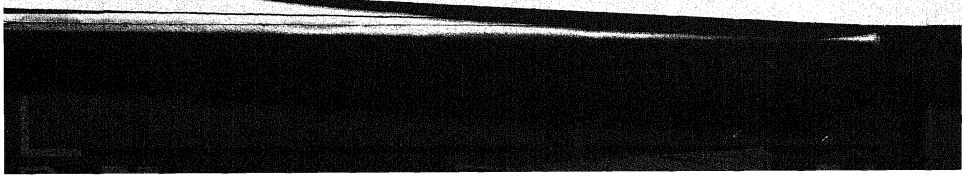

NON-DEHISCENCE OF ANTHERS IN PUNJAB-AMERICAN COTTONS.

BY

TREVOR TROUGHT, M.A.,

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(Received for publication on 23rd April 1928.)

The following preliminary note of a phenomenon which is at present under investigation at Lyallpur gives some details of observations made to date on the non-dehiscence of anthers in Punjab-American cottons.

O. F. Cook [1] has reported a case in Guatemala in 1905 where cotton, grown at a height of 2,600 feet above sea level failed to set any bolls on account of the non-dehiscence of anthers, and consequent lack of pollination, and ascribes this to the continuously humid and cool conditions which prevailed. Balls mentions on page 69 of "The Cotton Plant in Egypt" that non-pollination occurs " of course in cotton but it does not seem to be common under ordinary conditions." This non-pollination, however, probably was not due to non-dehiscence of anthers. No case of non-dehiscence was observed by the writer in Egypt, though cases of non-pollination were noted on still windless days in flowers with fully dehisced anthers. Kearney [2] who conducted extensive pollination experiments on American and Egyptian-American cotton also makes no record of the phenomenon in America. Barber [3] has reported a non-dehiscence of sugar-cane anthers. His paper gives the impression that this non-dehiscence may be due to an unsuitable climate, or incomplete acclimatisation of foreign canes.

The non-dehiscence in American cotton in the Punjab is, at certain times of the year, complete; every flower which opens at these times shows no dehiscence of any anther. Attention was first directed to this rather surprising condition by the fact that the shedding record in 1926 showed that on many days in June and July every flower which opened shed within the next few days. This was thought to be unusual and when looking for the cause of this, the non-dehiscence of anthers with the consequent non-fertilisation of the flowers appeared to provide the immediate

[1] Cook, O. F. The Causes of shedding in cotton. *Jour. Hered.* Vol. XII, 1921, p. 193.

[2] Kearney, T. H. Self Fertilisation and Cross Fertilisation in Pima Cotton. U. S. D. A. Dept. Bull. 1134, 1923.

[3] Barber, C. A. Studies in Indian Sugarcane, *Memoir Dept. of Agric. (Boat. Series)* Vol. 8, 1926, p. 109.

contributory cause. From the date of this observation, records were taken from time to time showing the progress of dehiscence; for it was obvious that, sooner or later if any crop were to be obtained, dehiscence must occur. During 1927 daily records have been taken as it was found that spasmodic records were not completely satisfactory.

One of the symptoms of the failure of Punjab-American cotton in 1926 was the poor development of seed and lint in many of those bolls which opened and it may be possible that the primary originating cause of this poor development of the seed and lint may have affected also the development of the pollen in the earlier part of the season. It is of interest to note that the records so far obtained for 1927 seem to show that the incidence of non-dehiscence was not so severe as in 1926.

Progress of dehiscence in normal anthers.

The normal progress of dehiscence was observed under a binocular dissecting microscope at the end of August. Continuous observation was maintained from 8 A.M. to 10 A.M. The anthers are kidney-shaped with two longitudinal pollen sacs, separated by a septum, and a central longitudinal suture. Dehiscence generally starts at one or both ends of the suture and proceeds slowly towards the centre of the anther. The progress of dehiscence is so slow that it cannot be followed by eye, but during the period of observation, the cleft gradually widened until the two thecae were completely separated. The pollen grains were seen to be regularly arranged within the cavity of the thecae at the time the two margins of the valves came apart.

The junction of the thecae does not always give way along the whole length of the anther. The two valves sometimes remain joined up at the centre. At this stage, whether separation is complete or not, the valves do not show any reflection on themselves, though by evening they are generally completely reflexed. Pollen protrudes immediately from the fully opened thecae and the movement gives the impression of a slow "welling up" from the cavity. The grains creep over the edges of the valve margin. The same effect is observed in those anthers which do not split completely. The fact that the valves are still joined at the centre does not prevent the protrusion of the pollen grains. In the case of two particular anthers which were observed continuously from 8 A.M. to 11 A.M. on the 6th September, the first anther was nearly fully opened, only being joined just at centre of the two valves. The second anther had only a small opening at one end. The width of these openings only increased very slightly if at all (*no* increase could be observed by eye during the time of observation). Pollen grains however welled up and overflowed as in a normally opened anther.

The mechanism of anther dehiscence in cotton has not been definitely determined, but it seems doubtful whether it can be explained entirely on the generally accepted lines of anther dehiscence (See the text books of Sachs, Jost, etc.). For example, in addition to the evidence of the above observations, when undehisced

anthers from fully opened flowers were split with a very fine needle along the line of dehiscence, the valves did not fly back as would be expected if any strain in the cell walls had been set up. Second, two flowers which opened in an artificially dried atmosphere did not show dehiscence. Third, from a preliminary examination of regular records of anthers dehiscence taken in 1927, it appears that the time of dehiscence did not depend on the humidity of the atmosphere, as a majority of flowers had dehisced anthers at a time when hygrograph records showed the humidity to be at or about its maximum. Fourth, cases of split anthers with the valves still incurved were frequently observed. On the other hand, when partially opened anthers are placed in Carnoy's fluid there is an immediate springing apart of the valves. Carnoy's fluid would, presumably, extract water from the anther tissues at once.

The anther wall has an inner layer of cells which is differentially thickened and is typical of tissue which is adapted for hygroscopic movement (See Fig. 226, p. 556 of Haberland's Physiological Plant Anatomy, 1914). Thus, though the original dehiscence may not be brought about by hygroscopic action, it appears that hygroscopic action completes the opening of the valves. As suggested to the writer by Dr. Lawrence Balls, the " welling up " of the pollen grains could then be explained by the slow contraction of the anther wall pushing the grains up and out of the cavity.

Distribution.

Regular observations were only made at Lyallpur, but casual observations in 1926 showed the phenomenon to be present at Risalawala 5 miles from Lyallpur, in ordinary zemindar's cotton near Lyallpur, at Okara in the Montgomery District and at Khanewal in the Multan District. Further observations in 1927 show the condition to be widespread, and that the percentage of non-dehisced flowers at different localities is generally of the same order of magnitude as at Lyallpur on any day.

It has been observed in 4F, 285F, 289F Punjab-American types and an Egyptian variety grown at Lyallpur and in different pure line strains of Punjab-American cotton growing at Lyallpur. It is not, therefore, confined to one variety though the records show that different varieties show the effect to different degrees. It seems very probable for example that 285F is more susceptible than 4F. This difference in varieties lends colour to the suggestion that the original cause of non-dehiscence may be the same as that which ultimately caused the 1926 failure. 4F, being a ' hardier ' variety than 285F, did not seem to be affected to the same degree as 285F.

Non-dehiscence has not been observed to occur in *desi* [Indian] cottons.

Appearance of non-dehisced anthers.

The anthers in many cases appear quite normal and it is not possible to distinguish by eye before dehiscence, those anthers which will later dehisce from those

which will not dehisce. Several flowers, however, show anthers which have a wrinkled appearance or in which the epidermis over the anther cavity is definitely sunken.

There also appears to be some difference in the structure of the connective of non-dehisced and dehisced anthers which frequently show differences in colour reaction when fixed in Carnoy's. This, however, is not invariable and may be accidental.

Pollen observations.

Anthers were preserved in alcohol and examined under the microscope and it has been found in those so far examined that, in every case, the non-dehisced anthers contain pollen which is not fully developed in some way. Generally it is found that the external wall of the pollen grain is fully developed, but the protoplasmic contents of the grain are lacking. The grain takes the form which would be taken by a punctured soft indiarubber ball when "dunched" in. Barber (*loc. cit.*) found that non-dehisced anthers of sugarcane contained an undeveloped mass of pollen mother cells.

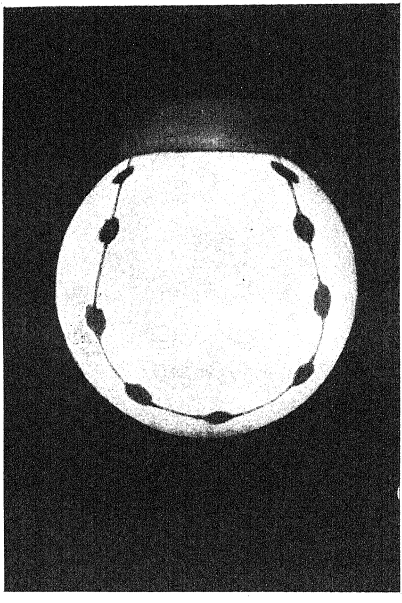
Denham [1] says that in the early stages of the growth of the pollen grain this appearance is observed. Material to examine this point is being collected to find out at what stage the growth of the pollen grain is arrested.

In the course of attempts to work out a staining technique for pollen grains, it was found that staining the anther in bulk with safranin, and counterstaining with light green in clove oil frequently gave a very clear differentiation of the pores, which took the light green. The technique of this double staining is not yet very precise. This differential staining enabled an interesting observation to be made on the arrangement of the pores in the grains. In all cases where the differential staining was clearly defined, it was found that the pores were arranged in a regular figure on the surface of the grain. This figure had a dumb-bell or open figure of eight outline and followed precisely the line of the seam found in the ordinary two piece covering of a tennis ball (Plate I). The number of pores was not constant but varied from 16 to 24. A similar arrangement of pores was found in the pollen grains of *desi* cotton with, however, fewer pores.

Denham, in his figure of a transverse section of a pore, refers to the 'pellicle' of the protoplasm as being immediately subjacent to the pore opening. When the pollen grain, differentially stained as mentioned above, is gently squashed so that the extine is ruptured, the protoplasmic contents separate from the outer coverings, and it is seen that this 'pellicle' is separate from the protoplasm and is stained by the light green. This membrane is part of the external coat and closes the pore opening. This has been confirmed by an examination of sections of pollen grains.

[1] Denham, H. J. The Cytology of the Cotton Plant. *Shirley Institute Memoirs*, Vol. III, 1924, p. 227.

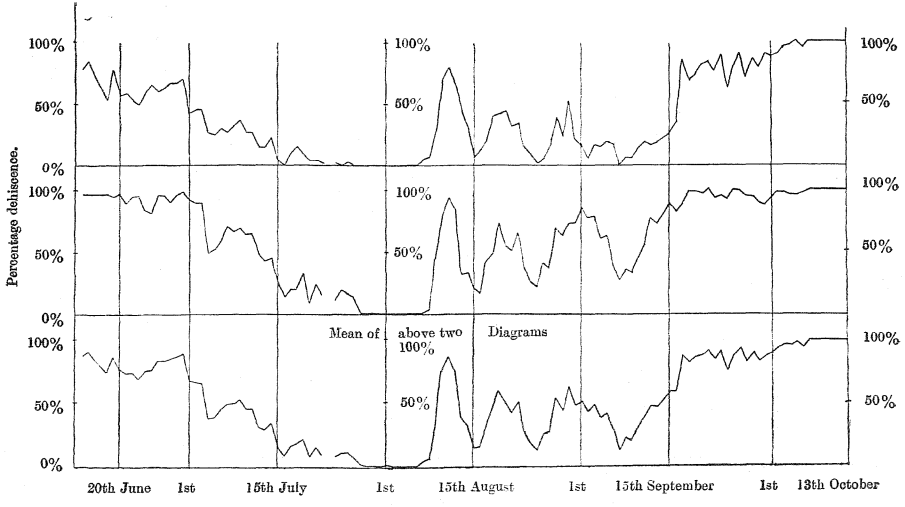
PLATE I.



Representation of the arrangement of pores on a pollen grain of 4F. Cotton.

PLATE II.

Diagram showing the course of anther dehiscence in 1927 for 4F and 285F Cottons.



The pores therefore do not open direct to the cell contents but are merely openings in the extine.

Denham states further that during the ripening of the anthers the pollen grains grow in size.

In view of the observations on anther dehiscence noted above, the fact that non-dehisced anthers contained pollen which was shrivelled and had apparently received a check to its growth, suggested that the first cause of dehiscence might be connected with this growth of the pollen grain. Actual measurements made on pollen grains so far show only that there are considerable differences between single anthers collected at the same time from the same flower. Further work is necessary, taking precautions to ensure that the cover slip cannot press on the grains, before a comparison of measurements between grains on different slides can be considered satisfactory. There are however indications that this growth does occur, and continues up to the dehiscence of the anthers.

1927 Observations.

Diagrams showing the percentage dehiscence of 4F and 285F cottons are reproduced in Plate II.

The curves are from records on approximately 40 flowers examined at random daily in the different varieties, the number of flowers showing dehiscence and non-dehiscence being recorded separately.

The fall of the curve is very similar for this full series of observations to the curve obtained in 1926 from more spasmodic observations. It is intended to correlate this curve at the end of the season with the plant development curves and meteorological data curves which are taken as a regular routine of the station. It is interesting to note here, however, that the daily variations correspond to some extent with the daily increase in height records. Also there appears to be some general detrimental factor operating from the middle of June to the end of July. Conditions favourable to growth appear to favour dehiscence and conditions unfavourable to growth increase the amount of non-dehiscence. The period of complete non-dehiscence corresponds with the period when daily flowering is at a minimum.

Further records on different varieties, and on 4F plots which have been subjected to different manurial and cultural treatments, all show little variation in percentage of non-dehiscence either on account of manurial treatment or culture. Locality and variety exercise some effect on the amplitude of the curves, but the daily fluctuations and general shape of the curves do not appear to depend on available food in the soil or on available water.

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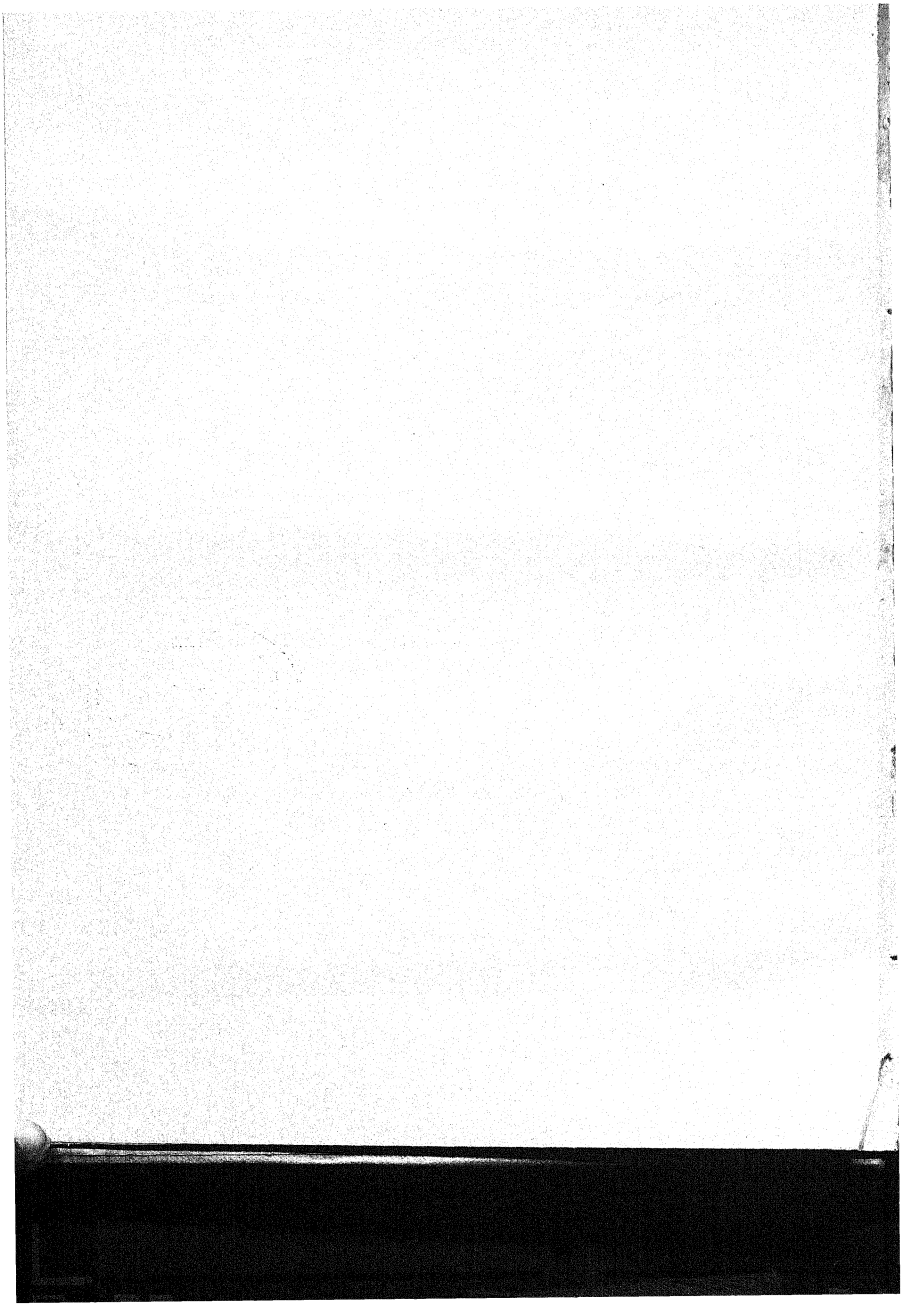


CONTENTS

STUDIES IN THE WILT DISEASE OF COTTON IN THE BOMBAY KARNATAK, SERIES 1.

BY

G. S. KULKARNI, M.Ag.,

AND

B. B. MUNDKUR, M.A.

(Received for publication on 16th April, 1928.)

I. INTRODUCTION.

BY

HAROLD H. MANN, D.Sc.

In every country where cotton is grown, the crop suffers to a greater or less extent, from loss due to death of the plants in a manner which is generally classed as *Wilt*. The most obvious symptom in the affected individuals is the gradual drooping and then withering, either of the whole plant or of a particular branch. This drooping generally appears first in the lowest or oldest leaves, and gradually proceeds upward until all leaves droop and die, and, in most cases, the whole plant, or the branch affected, withers, turns yellow and finally brown, and is lost. The plants affected in this way may succumb at any stage of their growth, from seedlings barely two weeks old to adult plants. Gradual death in the manner described is usual, but very rapid mortality is by no means uncommon. Plants where the main stem is attacked, whose leaves have shed and whose branches are killed, often throw out new shoots from near the base, which grow to entirety, though such individuals can generally be recognised by their dwarfed and bushy appearance.

Appearances such as those described are found, as already stated, in all countries growing cotton, and in many areas in India, form one of the most serious diseases of the crop. The extent of damage naturally varies from area to area, but it is considerable in all the cotton districts of the Bombay Presidency. Certain fields are always more affected than others, and in these it is not uncommon to find anything from 20 to 60 per cent. of the plants dead. In Gujarat, more particularly

in Broach, some of the heavily affected areas may show up to 20 per cent. loss of the crop. In Khandesh, in some fields, a destruction of from 30 to 40 per cent. is not uncommon, while in the Karnatak, the damage is perhaps greater than anywhere else, and fields are numerous where as much as 60 per cent. of the plants have been lost.

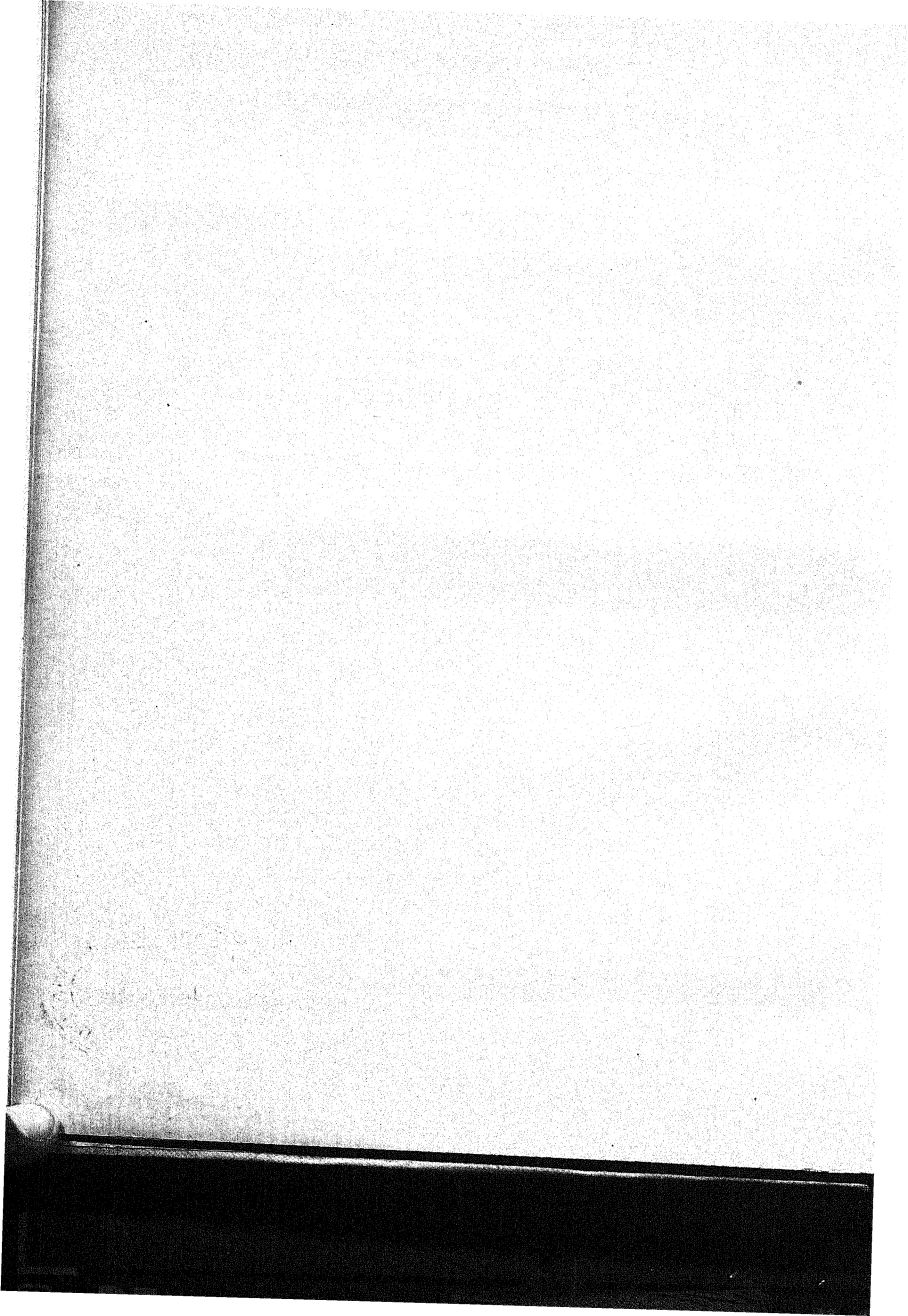
The actual loss has not, however, been exactly determined, but various estimates have been made. Butler [1] estimated a one per cent. loss over the whole country in 1912, due to wilt, but the basis of this estimate hardly seems sufficient for full reliance to be placed on it. The actual loss, in certain areas of the Central Provinces, from wilts, based on careful local observations at various periods, was recorded by Ajrekar and Bal [2] in 1921. They state that in 1907, there was a loss of 5 per cent. of plants in the Sauner District, on estimates made by Evans. In the following year (1908), Butler found 30 per cent. loss on an area of 80 acres near Akola (Berars). In 1911-12, Clouston determined the amount in five villages near Yeotmal as 33 per cent., in four villages near Amraoti (Berars) at 25 per cent, in three villages near Akola at 18 per cent., and in a village in the Buldana District, at 10 per cent. In 1919 an area under *roseum* and *gaorani* cotton in the Ellichpur District lost 47 per cent. of plants. While, in the same year, in a village named Kandly where *bishnoor jari* cotton was grown, the percentage of loss came to 15. The latest figures for the whole of the Bombay Presidency, from figures supplied by G. S. Kulkarni, indicate that the average loss cannot be less than 5 per cent.

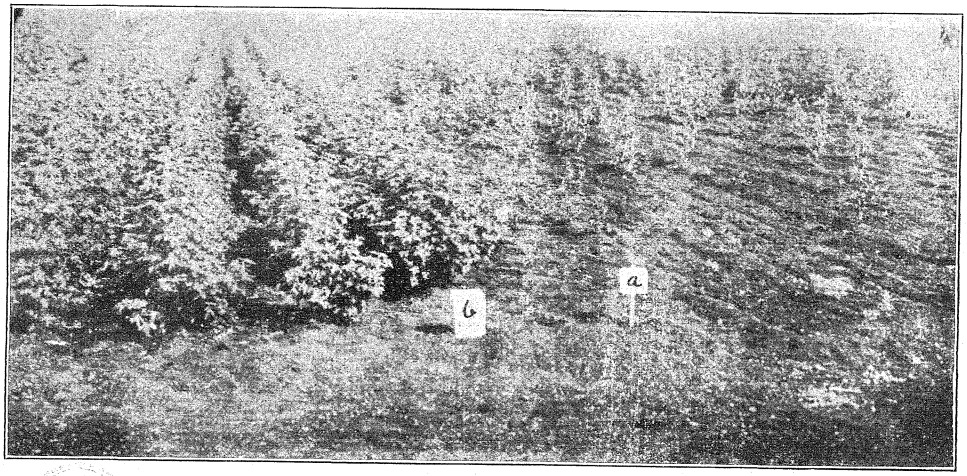
All those who have studied the subject agree that the damage is rapidly increasing. Kottur [3], for instance, after long experience of cotton in the Southern Maratha Country, notes in 1924 that " Observations indicate that wilt is extending " in that area. Dastur [4], again in 1924, states that it has spread to an alarming extent and is continuing to spread much in the Central Provinces and Berars. This being the case, the importance of the investigation of these wilt diseases is sufficiently obvious. Hence, in 1923, I placed a proposal before the Indian Central Cotton Committee that they should finance a thorough investigation into wilt of cotton in the Bombay Presidency, to be conducted in the area which was most notorious for the disease, namely the Bombay Karnatak. The Committee agreed to do so and the Bombay Government provided land and laboratory accommodation on the Government Experimental Farm (Station) at Dharwar. Mr. G. S. Kulkarni, whose work on various crop diseases in the Bombay Presidency was already well known, was selected to take charge of the actual inquiry which was commenced in September 1923 and has thus been going on for four and half years. The series of memoirs

[1] Butler, E J. *Report Agri. Res. Institute, Pusa* for 1913-14.
[2] Ajrekar, S. L., and Bal, D. V. Wilt disease of cotton in the Central Provinces. *Agri. Jour. of India*, Vol. XVI, 1921, p. 598.
[3] Kottur, G. L. Cotton Wilt in the Southern Maratha Country. *Agri. Jour. of India*, Vol. XIX, p. 155, (1924).
[4] Dastur, J. F. Investigation of Cotton Wilt in the Central Provinces. *Agri. Jour. of India*, Vol. XIX, (1924), p. 251.

to which the present note is an introduction include the data which have been obtained and the general results which have been secured.

At this stage, however, attention may well be drawn to the fact that the destruction of cotton plants in the manner already described is certainly not due to a single cause or even is not associated with any *one* agent. Ajrekar and Bal (*loc-cit.*) expressly caution themselves against taking it for granted that all wilted plants are due to a particular fungus, and call attention to the very widespread loss, with almost exactly similar external symptoms, associated with the cotton stem borer (*Sphemptera gossypii*). Again, in Sind, where wilting of the plants is not uncommon, the most common associated agency seems to be a *Rhizoctonia* fungus. But the organism most frequently associated with the disease in the greater part of the Bombay Presidency, is a species of *Fusarium*, which cannot be differentiated morphologically from a fungus discovered in Alabama (U. S. A.) in 1892. It is the particular form of wilt that is associated with this fungus that has occupied exclusively the attention of Mr. Kulkarni and his staff in the last five years. Though the association of the cotton plant and the fungus is constant, yet the relationship between them has remained very uncertain. The necessity of trying to clear up the nature of this relationship has guided a great deal of the work to be recorded in the present and succeeding memoirs, but it has not prevented attention being given to other methods of attack, such as the breeding of resistant strains of cotton suited for the area, of which an account will be given in its place.





The right side (*a*) shows the patchy nature of the cotton crop heavily infected with wilt. The left side (*b*) is a picture of a uniform crop free from wilt.

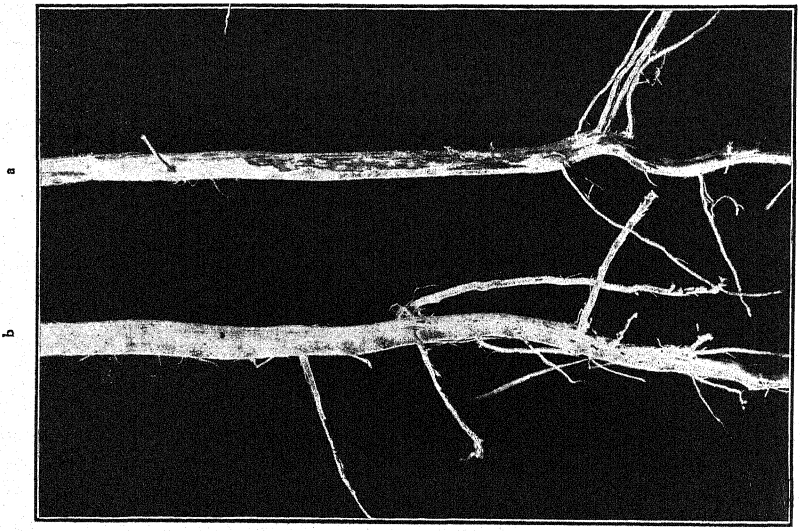


Fig. 2.

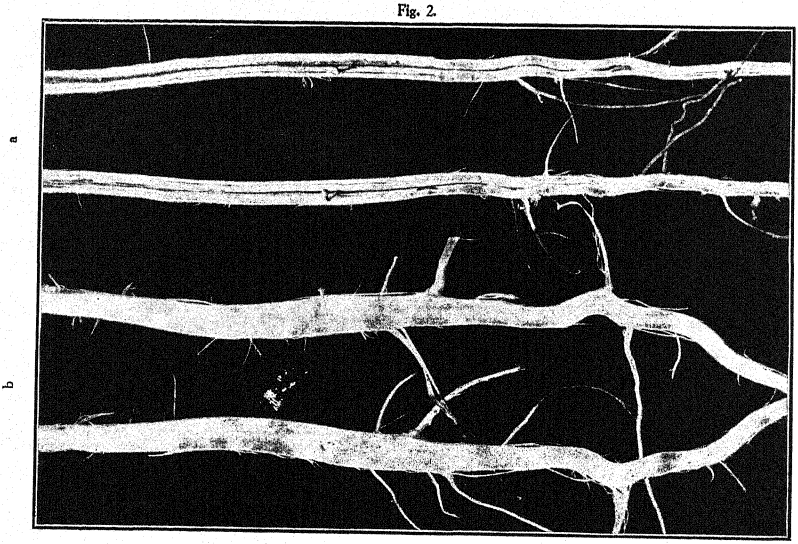


Fig. 1. (*a*) An infected plant showing dark streaks in the wood. (*b*) A healthy plant showing white surface of the wood.
Fig. 2. (*a*) Split-open stem of an infected plant showing discolouration of the vascular bundles. (*b*) Split-open stem of healthy plant showing white surface without any discolouration.

II. THE PARASITISM OF THE FUSARIUM ASSOCIATED WITH THE WILT DISEASE OF COTTON.

BY

G. S. KULKARNI, M.Ag.

It will be well to commence the present account of experiments to determine the parasitism of the *Fusarium* fungus found constantly associated with the wilt disease of cotton in the Southern Maratha Country (Bombay Karnatak) by a more detailed account of the symptoms of the disease as they occur in the area in question.

The most obvious sign of wilt in the affected cotton plants is the drooping and wilting of the leaves which generally proceeds from the base upwards, either on a branch or on the whole plant,—the shoot or plant affected finally hanging down. The withered leaves may become yellow and then brown, by which time the plant is dead. Plants may succumb at any stage of their growth, from seedlings barely two weeks old to adult plants. While gradual withering is the rule, plants which have wilted suddenly are not uncommon. Plants partially attacked are of frequent occurrence. Plants completely attacked, whose leaves are shed and branches killed, often recover by the development of new shoots from the base, but such plants can be generally recognised by their dwarfed and bushy appearance. In fields where wilt attack is of recent origin, a few withered plants will usually be found soon after the seedlings come up,—and owing to their small size, these usually escape notice. They, however, serve as starting points of wilt affected patches. In the following crop, a somewhat larger number of plants will generally be found to die round such centres, and gradually the patches are thus widened in successive crops,—so that in a few years they cannot be ignored. Large patches of this character are found in all heavily affected fields (Plate I). It would thus appear that the disease may often occur long before it is likely to be noticed by the actual grower of the cotton.

Seedlings in advanced stages of attack often show at the base of the stem, a discoloration of the bark which extends on to the tap root. In fully developed plants, however, no such discoloration is noticed, but on removing the bark, the tissues below, both at the base of the stem and on the upper roots, are always found blackened in streaks or in strips (Plate II, fig. 1, *a*). Such stems or roots, when split open, show dark streaks along the vascular strands (Plate II, fig. 2). These streaks, in advanced cases of attack, can be traced not only in the base of the main stem and tap root, but also higher up in the stem and branches, and also in most of the side roots.

The microscopic study of affected roots shows the following characters. In the early stages of attack there is a yellowing of the vessels and of the surrounding tissue. Later on this colour may change to brown and occasionally to dark brown. Many of such coloured vessels contain the hyphae of a fungus which at times completely fill them (Plate III, fig. 1). Fungus hyphae, however, may be found also in vessels apparently normal, that is to say, which do not show any discoloration. On the other hand, many affected vessels are filled, only with dark gummy masses. In the larger plants, similar fungus hyphae are found not only in the darkened portion of the root and stem, but can be traced much higher up the stem and branches and even in the fruit pedicels. The connection of this fungus which is so constantly associated with the disease as it occurs in the Bombay Karnatak has hitherto been doubtful, and the present study consists in an attempt to determine whether it is truly parasitic on the cotton plant,—its exact relationship to wilt attack in cotton being left for consideration in a later section of the present series of memoirs.

Fusarium fungus associated with cotton wilt.

The fungus was first described at Alabama in the United States of America in connection with the cotton disease, "*Frenching*," by Atkinson [1] who named it *Fusarium vasinfectum*. His inoculation experiments were few and were not very convincing. Smith [2] in his laborious work on the wilt diseases of cotton, water melon, and the cow pea, renamed the fungus as *Neocosmospora vasinfecta*, but the pathogenicity of the fungus was not proved, as his inoculation tests on cotton plants were not successful. He apparently confused the wilt *Fusarium* with *Neocosmospora vasinfecta* which is a saprophyte.

Later on the problem was taken up by Orton [3] who was able to produce the disease in healthy plants by inoculating the soil in which they grew, with a pure culture of the fungus. His results were based, however, on only one experiment and the number of plants treated was also small.

Recently Elliot in Arkansas [4] and Britton Jones [5] in Egypt have brought forward strong evidence of the parasitic nature of the *Fusarium* originally discovered by Atkinson. They have, in their investigation, dealt with a very large number of trials which leave no doubt as to the close association of the *Fusarium* with the wilt disease and of inoculation with the fungus being capable of producing the disease in healthy plants.

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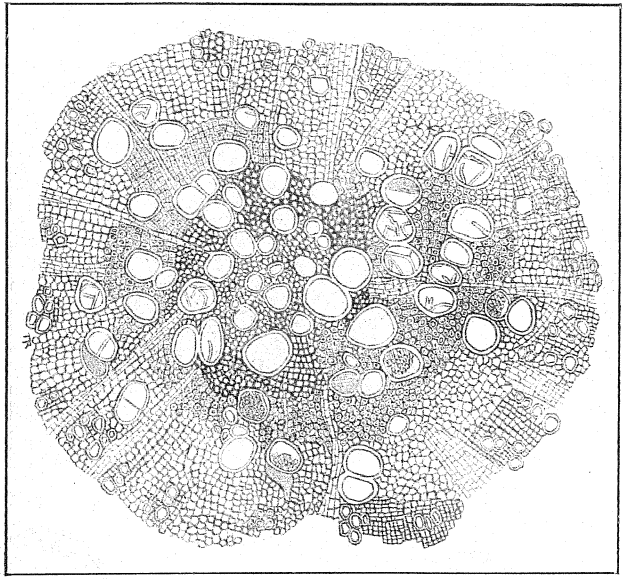
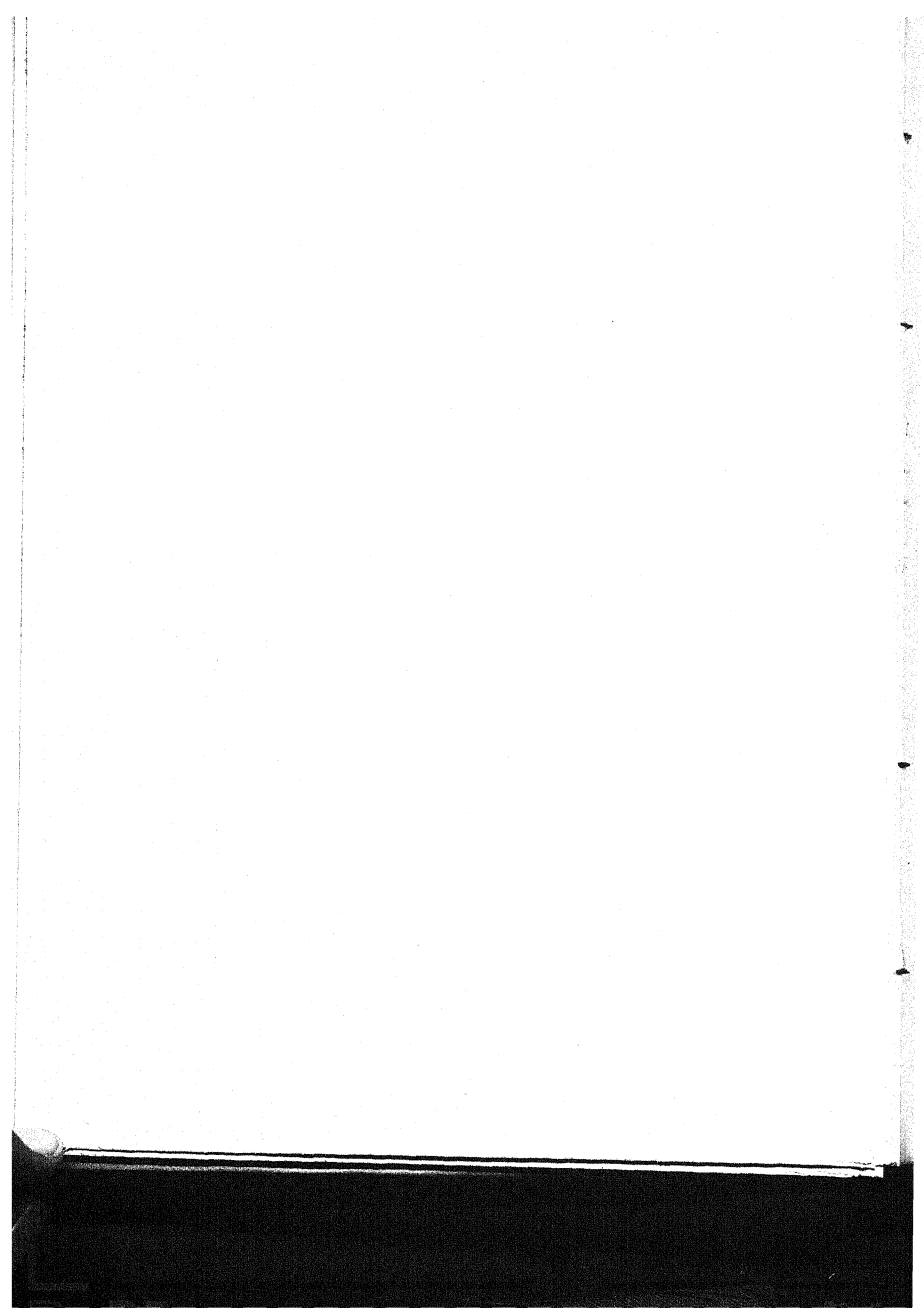
[1] Atkinson, G. F. Some diseases of cotton. *Ala. Agri. Expt. Sta. Bull*, No. 41, 1892.
[2] Smith Erwin, F. Wilt disease of cotton, water melon and cow pea. *U. S. Dept. Agri. Div. Veg. Path. Bull.*, 17, 1899.
[3] Orton, W. A. The Wilt disease of cotton and its control. *U. S. Dept. Agri. Div. Veg. Path. Bull.* 27, 1900.
[4] Elliot, J. A. Cotton wilt, a seed borne disease. *Journ. Agri. Res.* Vol. XXIII, 1923.
[5] Britton-Jones, H. R. Mycological work in Egypt during 1920-22. *Min. Agri. Egypt. Tech. Sci. Bull.* 49, 1925.



1. Cross-section of an affected cotton stem showing hyphae in some vessels.

2. One pot not inoculated with *Fusarium* (control).

PLATE III.



In India the earliest work on cotton wilt was done by Butler.[1] From the results of his few experiments he has declared himself quite confident of the parasitism of the wilt *Fusarium*. Recently he has given[2] the details of his experiments with photographic evidence and says "The percentage of deaths from wilt in the inoculated pots was low and the incidence of the disease was irregular, some plants escaping altogether while others succumbed, though all must be considered to have been exposed to the same intensity of infection. Nevertheless, those that became infected died in the manner characteristic of the *Fusarium* wilts, the examination of a larger number of wilted plants in the field supported this conclusion, and *the capricious results of the inoculations would appear to be due to the action of some factor which aids or hinders infection by the fungus.*"

In 1920 Ajrekar and Bal[3] took up the question afresh at Nagpur. They too in their inoculation experiments do not seem to have succeeded in getting uniform results and this irregularity they attribute to the advanced season when the experiments were conducted. The incomplete results thus obtained led them also to believe that the fungus was not a virulent parasite. But in a later communication Ajrekar[4] narrates details of experiments to prove the parasitism of the *Fusarium*.

More recently Dastur[5] has endeavoured to throw doubt on the *Fusarium* associated with cotton wilt as the cause of the wilt disease. His failure to produce disease in plants by inoculation with the fungus isolated from the wilted plants led him to look for other causes. He suggests and gives many experiments to support the view, that the wilt of cotton, in the Central Provinces and the Berars at any rate, is brought about by the absorption of compounds of iron and aluminium from the soil, that the *Fusarium* may be merely a contributory factor in hastening the death of the plants, and that the fungus follows in the wake of accumulation of these compounds in the tissues.

It is thus obvious that the evidence available on the parasitic nature of the cotton wilt *Fusarium* is scanty and conflicting. It, therefore, seemed desirable to investigate the conditions under which the *Fusarium* is able to attack the cotton plants.

Experimental Work.

Series 1. To test the parasitism of the *Fusarium*, a series of infection experiments were carried out with a pure culture of the fungus. The culture used was obtained as follows. A wilting cotton plant about a month old was uprooted from the field, the root was well washed, and the presence of internal mycelium was ascertained

[1] Butler, E. J. *Rept. Agri. Res. Inst., Pusa* for 1913-14.
[2] Butler, E. J. The Wilt disease of cotton and sesamum in India. *Agri. Jour. India*, Vol. XXI, 1926.
[3] Ajrekar, S. L., and Bal, D. V. Observations on the wilt disease of cotton in the Central Provinces. *Agri. Jour. India*, Vol. XVI, 1921, p. 598.
[4] Ajrekar, S. L. The cause of cotton wilt in India. *Jour. India Bot. Soc.*, Vol. V, 1926.
[5] Dastur, J. F. A preliminary account of the investigation of cotton wilt in the Central Provinces and Berar. *Agri. Jour. of India*, Vol. XIX, 1924, p. 251.

by microscopic examination. With a sterile knife a small piece of it was cut, dipped in alcohol, flamed and was immediately dropped in a sterile moist chamber consisting of a watch glass in a petri dish. The piece thus treated gave rise to white mycelium filaments bearing spores (*microconidia*) at both the cut ends in three days. The fungus was plated and from one of the little colonies, a culture was made on glucose agar tube on August 30th, 1923. The plants were grown in pots washed with a strong solution of copper sulphate. The earth was sterilised in the autoclave at 20 lb. pressure for half an hour. The seed was delinted with strong sulphuric acid and was again sterilised, prior to sowing with mercuric chloride solution. The inoculation was made *just before sowing the seed* by pouring the culture of one tube, mixed with a sufficient quantity of water, on the surface of the soil in each pot. Twenty seeds were put in each pot. The results were as follows.

No. of pots.	Treatment	Date of sowing	Results
1 to 28	The surface soil was inoculated	20th Oct. 1923 . .	No deaths up to February 1924.
29 to 30	Control pots	20th Oct. 1923 . .	No deaths.

The failure of these inoculation tests was complete. The experiments were terminated by the end of February and no further trials were made as the hot weather had set in.

Series II. On August 3rd, 1924, fourteen pots, out of the twenty-eight that were inoculated in 1923, were inoculated a second time without sterilising the soil again. The fungus used was a sub-culture on rice of the culture used in Series I. The inoculation was made by mixing thoroughly the culture diluted with water with the top four inches of the soil in pots and not by merely pouring it on the surface soil as was done previously and then, at once, sowing the seed prepared as before. This time the inoculation was partially successful as most of the plants wilted. Wilting commenced on August 11th, 1924 (that is, after eight days) and deaths increased week by week until the position on October 9th (that is, after 68 days) was as follows.

Pot No.	No. of plants	Dead plants on 9th Oct. 1924	Results
1	10	8	The two living plants were stunted.
2	,,	4	
3	,,	6	
4	,,	10	
5	,,	8	

Plot No.	No. of plants	Dead plants on 9th Oct. 1924	Results
6	10	10	
7	,,	4	
8	,,	10	
9	,,	10	
10	,,	10	
11	,,	10	
12	,,	10	
13	,,	10	
14	,,	10	
29	,,	*Nil.*	The controls where no deaths took place.
30	,,	*Nil.*	

The success of the inoculation leading to the death of the plants in the inoculated pots has been complete in nine out of fourteen cases. In the others, some of the plants have died, with all the appearance of wilt, in every case, the percentage varying from 40 per cent. (pots 2 and 7) to 80 per cent. (pots 1 and 5). In one of the latter cases the living plants were very stunted.

Two questions at once arose. Why is it that a number of plants have been able to survive and why is it that inoculation in the present case has produced the wilting of a large number of the plants in typical fashion, whereas the previous experiment gave no results? For the present, attention may be concentrated on the second point.

Series III. It seemed likely that the complete failure of the first series of inoculations might be at least in part, due to the method adopted, of pouring the fungus culture over the surface of the soil, as under these conditions the parasite might have little chance of reaching the growing roots. Various methods of inoculation were therefore adopted with results as follows. The inoculation used in these experiments was a sub-culture of that used in Series II.

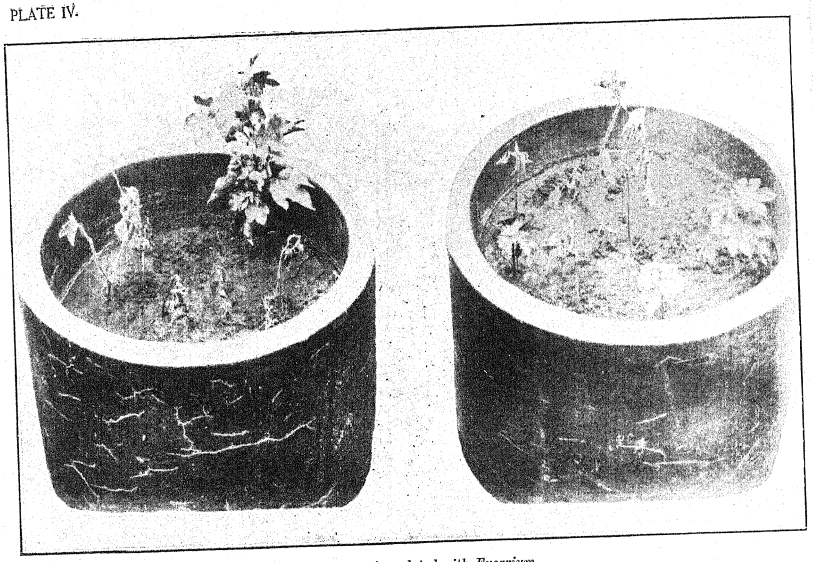
Pot No.	Treatment	Date of sowing	Results
10	Fungus mixed with the first four inches of soil	15th Aug. 1924 . .	8 out of 15 had died on 14th November 1924.
12	Ditto. . .	Ditto. .	10 out of 15 had died on 14th November 1924.
9	Control . . .	Ditto. .	None died.

Pot No.	Treatment	Date of sowing	Results
201	Fungus mixed throughout the soil.	16th Aug. 1924 . .	14 out of 15 had died on 6th September 1924.
202	Ditto .	Ditto .	All dead.
203	Ditto. .	Ditto .	All dead.
204	Control	Ditto .	All 15 were living.
209	Fungus mixed with top layer when plants were 15 days old.	15th Oct. 1924 . .	None out of 15 had died even on 5th November 1924.
209*	Ditto .	Ditto .	Ditto.
210	Control	Ditto .	Ditto.
206	Inoculation was done by mixing wilted plant material with Soil.	16th Aug. 1924 . .	11 out of 15 were dead on 10th September 1924.
208	Ditto .	Ditto .	12 out of 15 were dead.
205	Control	Ditto .	No deaths.

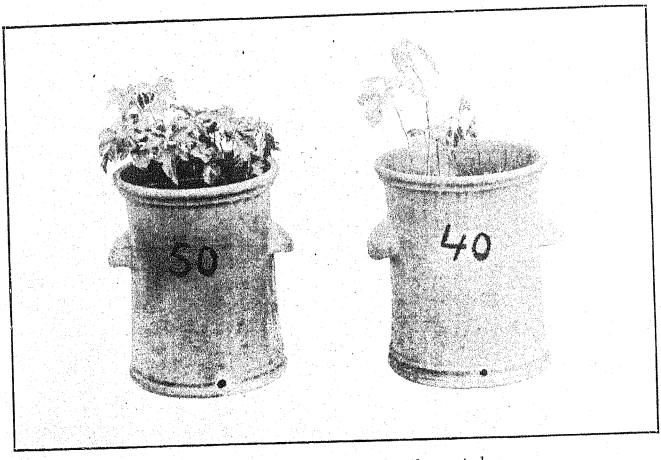
In these experiments, the dead plants showed typical wilting, and, on sectioning the characteristic browning of the vessels, some of which contained hyphae, was noticed. A culture of *Fusarium* fungus was obtained from these plants which on comparison with original fungus was found to be morphologically identical. It is thus evident that to get consistently successful results of infection experiments, the inoculation must be done by mixing the culture with the entire mass of soil before potting; the process of inoculating the soil during the growth of the plants is a failure. Furthermore wilted plant material will serve as a good means of inoculating the soil.

Series IV. The culture that was used in these experiments was obtained from a wilted plant from the field. The usual process of isolating the fungus was gone through on October 6th, 1924. The fungus appeared on October 9th, and a single spore isolation was made on the 12th of the same month. When the work was started in August 1925, this culture was used for the inoculation experiments. The soil was sterilised in the autoclave, the pots washed with a strong copper sulphate solution and the fungus was mixed with the soil one week before the sowing

PLATE IV.



1. Two of the pots inoculated with *Fusarium*.



2. One pot inoculated and the other control.

was done. The fungus for inoculation was grown in * Richard's liquid medium and a generous quantity of the fungus was added to the soil. The following are the results.

Pot No.	Treatment	Date of sowing	Results
289	Fungus mixed on 10th August 1925.	17th Aug. 1925	Wilt noticed on 2nd September 1925. All 16 dead on 10th September 1925.
290	Ditto	Ditto	First death on 2nd September 1925 and 18 out of 19 dead on 10th September 1925.
291	Control	Ditto	All the 15 plants healthy.
292	Fungus mixed on 24th August 1925.	31st Aug. 1925	Deaths began on 19th September 1925. 15 out of 18 died on 1st October 1925.
293	Ditto	Ditto	14 out of 18 died on 1st October 1925, rest as in previous one.
294	Control	Ditto	All 15 healthy.
517	Fungus mixed on 4th October 1925.	11th Oct. 1925	First death on 25th November 1925. 7 out of 17 died by 11th December 1925.
518	Ditto	Ditto	13 out of 15 died by 11th December 1925.
519	Control	Ditto	No deaths, all 15 healthy.

(Plate III, fig. 2 and plate IV, fig. 1.)

Here also wilting was typical. In the pots number 289-290, 34 out of 35 had died, while in the pots numbers 292 and 293, 517 and 518, 29 and 19 had died out of total of 36 and 27 respectively. The number of deaths are not consistently similar, but though it is proportionally large, the mortality is in no case complete.

* Richard's medium :—

Potassium nitrate	10·00 grams.
Potassium biphosphate	5·00 „
Magnesium sulphate	2·50 „
Cane sugar	50·00 „
Water	1000·00 c. c.

Series V. The culture used in these experiments was a sub-culture of that used in the last series. Inoculation was done, as before, a week prior to sowing was done, and the results obtained are shown below.

No. of pots.	Treatment	Date of sowing	Results
18	Fungus mixed on 3rd July 1926.	10th July 1926 . .	First death on 27th July 1926, and 5 out of 10 on 4th August 1926.
19	Ditto .	Ditto .	9 out of 10 died on 4th August 1926.
22	Control	Ditto .	No deaths.
36	Fungus mixed on 30th July 1926.	5th Aug. 1926 . .	First death on 2nd September 1926, and 15 out of 20 on 22nd September 1925.
40	Ditto .	Ditto .	11 died out of 18 on 22nd September 1926. (Plate IV, fig. 2.)
50	Control	Ditto .	All 15 healthy.
96	Fungus mixed on 30th August 1926.	6th Sept. 1926. . .	First death on 1st November 1926 and on 1st January 1927, 4 out of 14 had died.
97	Ditto .	Ditto .	On 1st January 1927, 8 out of 14 died.
98	Control	Ditto .	No deaths.

These experiments gave further unmistakable evidence of the parasitic nature of *Fusarium*. The fewer deaths in some of the pots may be due to some environmental factor and this matter will be treated when the influence of soil factors is discussed.

Series VI. An experiment was tried to see whether soil in the field condition could be infected. A small plot quite separated from the main infected area and free from disease was chosen. The absence of the disease in the plot was ascertained by growing a susceptible type of cotton in the year 1924 when not a single wilt infected plant was noticed. In the year 1925 a small area, 20′ by 20′ of this plot was, infected with the sub-culture of the fungus used in Series IV. The inoculation was effected just before sowing the crop, by sprinkling on the surface of the soil a bucketful of water in which a culture of twenty tubes was mixed. Not a single case of wilt infection was noticed in that season. In the year 1926, the same

area was again infected two months before the sowing time. The infective material consisted of stems and roots of wilted plants collected in the year 1925. In addition to this a large quantity of the fungus grown in liquid culture was also mixed. The whole of the material was well worked out in the soil upto a depth of five inches. Wilt appeared in the crop and the wilted plants differed in no way with those that were found in wilt infected fields. The experiment is being continued and cotton is sown in the same area every year. The results so far noted are as under.

Year	Treatment	Results
1923-24 . .	None but susceptible cotton was sown	No wilt.
1924-25 . .	Small area infected	No wilt in affected area.
1925-26 . .	Additional infection	116 out of 527 died or about 22 per cent.
1926-27 . .	No special treatment . . .	48 out of 471 died or 10·2 per cent.

The experiments noted above are but a few of the several trials carried during the last four years on wilt investigation. All these pot experiments have given clear evidence that the *Fusarium* is able to cause cotton wilt under certain conditions *viz.* (1) that the experiments are carried on in the proper season, July to December, and that the infection of the soil is done sometime before the seed is sown. From February onwards inoculation tests have invariably given negative results.

Summary.

Cotton plants as grown in the Bombay Karnatak suffer very heavily from a wilt disease, which is associated with the presence of a fungus which is morphologically undistinguishable from the *Fusarium vasinfectum* Atk. This wilt disease causes a loss of about five per cent. of the crop grown in this part of the Bombay Presidency.

This wilt disease can be noticed in the fields from the time the plants are a few days old (less than two weeks), but the plants may be attacked at any age.

The problem has been to ascertain what is the connection of the *Fusarium* fungus with the wilt disease, and, firstly, to determine whether it is actually parasitic on the cotton plant or no. It seems now clear that the fungus is a definite parasite of certain varieties and strains of the cotton plant, that is to say, that when it is present in sufficient quantity in the soil, and when these conditions are favourable, the plants die, and the fungus can be obtained in pure condition from the tissues of the dead or dying plants.

In order to secure the success of such inoculation, it is necessary that the fungus should be present throughout the portion of the soil where feeding rootlets are likely to reach, and not merely be present in the superficial layer of the soil. Further,

the inoculation should be done before the seeds are sown and not during the growth of the plants. Further, once the soil has been inoculated, it remains infective for at least a year (Series VI).

In no case, however, was it possible to be sure that all the plants growing in presence of the fungus would be affected, or at least would die. The cause of this merely partial success of the parasitic fungus in killing the plants suggests that some other condition is necessary to enable the parasite to attack and destroy the cotton. Whether this is so, and, if so, what is the nature of this other condition will be discussed in a later section of the present series of memoirs.

III. THE PATHOGENY OF WILTING IN COTTON PLANTS.

BY

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AND

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Recent researches on the exact pathogenic action of parasitic fungi in producing wilt in plants have shown that many of these fungi secrete substances whose action on the living tissues of the plant is lethal, so that the water supply of the transpiring area is cut off and the plant commences to wilt. The mechanical clogging of the xylem tubes with which wilting was formerly associated does not appear to be a feature that would lead to the complete stopping of the transpiration current, for the anatomical examination of a large number of heavily infected plants has shown that the number of vessels that are clogged by the mycelium is not large and that in all the cases examined, there exist xylem ducts free from any infection.

Wilting associated with the presence of fungi of the *Fusarium* type, is characterised by a complete and rapid killing of the tissues, the cells being discoloured and a brown and a gummy substance being often found filling the lumen of the vessels. The plants may regenerate later on, for wilted plants left undisturbed in the fields have been found to produce new shoots, a dormant bud having evidently leapt into activity as soon as favourable conditions set in.

The possible connection of toxic substances, however produced, with the death of plants in wilting diseases was first pointed out by Peltier [1] who in 1912 determined that a Botrytis associated with wilting plants produced a "harmful substance" that "may be some organic acid other than oxalic or it may be a toxin of some kind which, however, is not destroyed by heating to 100.°C." In 1913 Hutchinson [2] obtained wilt in tobacco plants by injecting them with the precipitate from bouillon cultures of *Bacterium solanacearum*, secured by precipitation with alcohol and in 1916 Coons and Goss [3] showed that the filtrate from cultures of *Fusarium oxysporum*

[1] Peltier, G. L. Physiology and life-history of a parasitic Botrytis on pepper and lettuce. *Ann. Rept. Missouri Bot. Garden*, Vol. 23.

[2] Hutchinson, C. M. Rangpur Tobacco wilt. *Dept. Agri. India, Mem. Bot. Ser.*, Vol. I, pp. 67-83, 1912.

[3] Coons, G. H., and Goss, R. W. Rept. of the Botanist. *Rept. of Michigam Board of Agri.*, Vol. 55, 265-271, 1916.

was as effective as the parasite itself in causing the wilt of potato plants. Reference may further be made to the work of Brandes,[1] Dowson,[2] Bewley,[3] Fahmy,[4] Goss[5] and Barnum[6] who have proved the presence of substances injurious to plants in the filtrates of fungus cultures.

A very comprehensive study of the action of the filtrates from cultures of *Fusarium vasinfecta* Atk., on cotton plants has been made by Rosen[7] quite recently. He has been able to produce all the wilt symptoms in a comparatively short time with the filtrates of solutions provided with nitrogen in an inorganic form and in which the fungus had been growing for a period of about three weeks. In filtrates from culture solutions where nitrogen in an organic form had been supplied and where the fungus had grown for a similar period, wilting did not occur. The distillation products and the residue of the former filtrates were able to induce wilting, the residue having been found to be slightly more toxic. Rosen believes that the growth of the fungus for a considerable period results in the production of nitrites in the media which even in dilute doses are toxic to cotton plants.

Detailed study of the media in which *Fusarium lycopersici* had been growing has been made by White[8]. Evidence has been secured and brought to bear on the question of the production of injurious substances by fungi associated with various forms of wilt in the culture solutions in which they have been allowed to grow.

Experimental work.

The work reported in this paper was done in 1924 by the writers but the publication of the results was unavoidably delayed. The delay, however, has enabled them to further test their results in the light of Rosen's experiments and they have been able to partially confirm his results.

Materials. The fungus used in these experiments was isolated in 1924 and the parasitism of the culture has been proved (*see* part II).

Methods. The fungus was grown in a liquid medium for varying periods of time, at the end of which the cultures were freed from the mycelium by a preliminary

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[1] Brandes, E. W. Banana Wilt. *Phytopathology*, Vol. IX, 339-389, 1919.

[2] Dowson, W. J. On the symptoms of wilting of Michaelmas daisies produced by toxins secreted by Cephalosporium. *Trans. Brit. Myco. Soc.*, Vol. 7, 283-286, 1922.

[3] Bewley, W. F. The sleeping disease of tomatoes. *Ann. Applied Biol*, Vol. 9., 116.

[4] Fahmy, T. Production by *Fusarium solani* of an excretory substance capable of causing wilting in plants. *Phytopathology*, Vol. 13, 543-550, 1923.

[5] Goss, R. W. Potato stem end rot due to *Fusarium eumartii*. *Neb. Expt. Stat. Res. Bull.*, No. 28, 1-87, 1924.

[6] Barnum, C. C. Production of substances toxic to plants by *Penicilium expanse*. *Phytopathology*, Vol. 23, 1923.

[7] Sahasrabudhe, D. L., and Daji, J. A. Nitrogen recuperation in the Bombay Deccan, Part I. *Dept. Agri. India, Mem. chem. Series*, Vol. VIII, p. 67, 1925.

[8] White, R. P. Studies in *Fusarium lycopersici* which causes the wilt of Tomatoes. *Jour. Agri. Res.*, Vol. 34, 1927.

filteration through glass wool. The different treatments given to the filtrate are described under each experiment. The first few experiments were conducted using Medium A described below and later a modification of Richard's solution was alone used.

Medium A.—

	grams.
Ammonium phosphate	2·0
Potassium nitrate	2·0
Magnesium sulphate	0·25
Lactic acid	1·0
Cane sugar	30·0

Distilled water to make the above a litre.

Modified Richard's solution.—

Acid potassium phosphate	2·5
Potassium nitrate	5·0
Magnesium sulphate	1·25
Cane sugar	30·0

Water to make the above a litre.

The solutions were made by using Merck pure chemicals. Prior to inoculation by the fungus, they were sterilised at 15 lb. pressure for 15 minutes. The solutions did not induce any physiological wilting in cotton plants even after 72 hours. After sterilisation, the p^H value of the first solution was 6·1 and that of the second 4·6.

Experiment I. Forty c. c. of solution A were placed in each of the twenty flasks of 100 c.c. capacity. They were sterilised and inoculated as follows :—

5 flasks on 31st August 1924.
5 flasks on 7th September 1924.
5 flasks on 14th September 1924.
5 flasks on 21st September 1924.

On the 28th of September, the reaction of the medium in each flask was determined by using the colorimetric method of Gillespie. Filtrates of one, two, three and four weeks old cultures had 4·9, 7·0, 7·6 and 7·9 p^H respectively. One-half of the quantity of the solution in each case was boiled for five minutes. Vigorously growing plants of a susceptible variety of cotton (Dharwar 1, a pure strain of Kumpta variety, *Gossypium herbaceum* Linn.) which were about a month old were carefully uprooted, placed in a large dish of water and brought to the laboratory. They were separated from the roots by being cut under water. Excepting the uppermost tier of leaves,

all the other leaves were removed and the plants were placed in respective solutions at 10 A.M. The results are as tabulated below :—

TABLE I.

Action of filtrates on cotton plants.

No.	Age of filtrate	Time taken to wilt			
		Boiled	Unboiled	Boiled, half strength	Unboiled, half strength
		hrs.	hrs.	hrs.	hrs.
1	One week	6	6	6	5
2	Two weeks	5	5	6	6
3	Three weeks	4	4	4	4
4	Four weeks	3	3	3	3
5	Cut plants left in sterile solution did not wilt even after 72 hours.				
6	Placed in distilled water, cut plants did not wilt at all.				
7	Cut plants when placed in an empty test tube wilted in half an hour, but revived when placed in water immediately.				

It will be evident from the above table that filtrates of 4 weeks old cultures were more toxic than the rest and the dilution of the filtrates did not seem to reduce their toxicity appreciably. It was observed that wilting usually commenced from the lower leaves, but in the filtrates of older cultures, the shoots sank suddenly. When these plants were transferred to pure water, they did not revive, showing that the effect of the toxins present in the filtrates on the living tissues of the plant was of a permanent nature. The boiled filtrates were as toxic as the unboiled filtrates. The injurious substances in the filtrate were therefore thermostable and of non-enzymatic nature, as enzymes are known to be destroyed on boiling.

Experiment. 2. In order to determine whether the reaction of the filtrates had any influence on wilting produced, blank culture solutions were adjusted to 4·9, 7·0, 7·6, and 7·9 p^H, corresponding with the values of the one, two, three and four weeks old cultures, respectively. Cotton plants when cut as in the previous experiment and left in the solutions did not wilt.

Experiment. 3. The effect of the filtrates on a very highly resistant type of cotton (Gadag 1, a pure line selection from Dharwar American cotton, *G. hirsutum* Mill.) was next determined. The filtrate used was from a four weeks old culture and the cut plants left in full strength, boiled and unboiled, and half strength, boiled

and unboiled filtrates, wilted in about 3 to 7 hours. This showed that even an immune type of cotton plant could not resist the injurious effects of the substances secreted by the fungus in the culture.

Experiment 4. In determining the toxicity of the filtrates of staled *Fusarium* cultures, Rosen [1] used Richard's liquid medium. An experiment was, therefore, devised to determine whether in this medium the species of *Fusarium* used in these studies was able to secrete injurious substances. Preliminary experiments with cut cotton plants placed in Richard's solution showed that this highly concentrated solution would induce physiological wilting in plants. However, when it was diluted by the addition of an equal quantity of water, the plants did not wilt. 300 c. c. of the diluted solution were placed in one-thousand c. c. flasks, sterilised and inoculated. The culture was incubated for three weeks. At the end of this period filtrates were obtained and their effects on cut as well as whole plants of Dharwar 1, the susceptible variety, Dharwar 2, a resistant variety (pure line selection from Kumpta variety of cotton, *G. herbaceum*) and Gadag 1, the highly resistant variety of cotton, were determined. The effects of the filtrates sterilised for 10 minutes at 15 lb. pressure and those filtered using the filter candles were also determined in each case. The results are tabulated below :—

TABLE II.

Effect of staled Fusarium *filtrates on cotton plants.*

Filtrate	Dharwar 1		Dharwar 2		Gadag 1	
	Cut	Uncut	Cut	Uncut	Cut	Uncut
	hours	hours	hours	hours	hours	hours
Filtered through filter paper .	3	17	10	21	8	25
Sterilised filtrate. . .	5	23	17	25	17	25
Filtered through porcelain filter.	3	18	..	..	..	..
Filtered through paper, half strength.	7	17	..	..	..	..
Filtered through porcelain, half strength.	7	17	..	..	..	..
Blank solution . . .	No wilt.	No wilt.				
Blank solution adjusted to 5·7 pH *.	No wilt.					

(*pH value of the staled solution after fungus had grown for three weeks.)

[1] Sahasrabudhe, D. L., and Daji, J. A. Nitrogen recuperation in the Bombay Deccan, Part I. *Dept. Agri. India, Mem. Chem. Series*, Vol. VIII, p. 67, 1925.

It will be noted that the injurious effect of the filtrates on cut plants was more readily observed than on the whole plants. Filtrates obtained by filtering through filter paper and porcelain had almost identical toxic properties. Sterilisation of the filtrates did not alter the properties of the filtrates in any significant way. Filtrate of a culture solution in which *Fusarium udum* Butler, which causes the wilt of pigeon peas (*Cajanus indicus*) and which does not affect cotton, had grown produced wilt also in the cotton plants.

Chemical analysis of the filtrates. Fahmy [1] found that *Fusarium solani* produced an oxalate in the culture solution. The filtrates from cultures of this *Fusarium* did not show any oxalic acid. The presence of a lactate was suspected and it was observed that the addition of lactic acid to Medium A had led to the production of as much as 1·99 grams of it per litre. Experiments with a blank solution containing. 1·99 grams of lactic acid per litre showed that this acid or its salt was not of any consequence in producing wilt by the filtrate.

Rosen [2] found that in stale Richard's solution, the fungus had induced the formation of nitrates and he was able to detect 0·0125 to 0·04 mgm. of nitrite nitrogen per cubic centimeter. In the filtrates obtained here the quantity of nitrate formed was very minute, there being 0·025 mgm. of nitrite nitrogen per litre, as against 12·5 mgm. of nitrite nitrogen per litre obtained by Rosen. These tests were carried out in the laboratory of the Agricultural Chemist at Poona and the method used in the determination of the nitrite nitrogen is described by Sahasrabudhe and Daji [3]. When 0·025 mgm. of nitrite nitrogen in the form of sodium nitrite (0·0375 mgm.) were added to modified Richard's solution and when whole and cut cotton plants were left in the solution, no wilting occurred even after 48 hours. This last test was twice repeated with the same result. Tests for oxalic acid and lactic acid in these filtrates gave negative results also.

Recently Letcher and Willaman [4] have shown that the pathogenicity of different strains of *Fusarium lini* Bolley depends on their power of producing alcohol. It is possible that alcohol is, in the present case also, associated with the wilt of cotton plants due to *Fusarium vasinfectum*. Puri [5] has reported the toxic effect of both ethyl and methyl alcohol in very small concentrations towards barley plants. The presence of alcohol in the filtrates used in these experiments was noticed and further experiments along the line may possibly lead to at least a partial solution of the problem.

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[1] Fahmy, T. Production by *Fusarium solani* of an excretory substance capable of causing wilting in plants. *Phytopathology*, Vol. 13, 543-550, 1923.

[2] Sahasrabudhe, D. L., and Daji, J. A. Nitrogen recuperation in the Bombay Deccan, Part I. *Dept. Agri. India, Mem. Chem. Series*, Vol. VIII, p. 67, 1925.

[3] Letcher, L. W., and Willaman, J. J. Alcohol fermentation by *Fusarium lini*. *Phytopathology*, Vol. XVII, 1927.

[4] Puri, A. N. Effect of methyl and ethyl alcohol on the growth of barley plants. *Ann. Bot.*, Vol. XXXVIII, 745-752, 1924.

Conclusions and summary.

This study of the effect on cotton plants of solutions in which *Fusarium vasinfectum* Atkinson, so closely associated with the wilt disease of cotton, had grown, leads to the conclusion, confirmed by microscopic evidence, that the primary factor leading to the death of cotton plants is not clogging of the vascular ducts by the mycelium of the fungus.

The active factor causing wilt in cotton plants used in these experiments appears to be a chemical compound or compounds occurring in the liquid in which the fungus has grown, which is not destroyed by boiling and which is not removable by filtration through porcelain filters. It is not, moreover, destroyed by heating the filtrates in an autoclave at 110° to 115° C. The nature of the substance or substances has not been determined, but lactic and oxalic acids are definitely excluded and nitrites do not appear to be the cause of the result noted. The solutions in which the *Fusarium* had grown were not only fatal to susceptible type of cotton plants but they also caused wilt symptoms in resistant types and even in types (like Gagad 1) which are highly resistant and even be considered as immune.

MGIPC—M—III-1-76—27-11-28—650.

ACKNOWLEDGMENTS.

Extensive work on this problem was made possible by the generous financial help given by the Indian Central Cotton Committee. An annual grant of Rs. 3,000 was sanctioned for five years and salaries for two temporary assistants were provided for three years. My acknowledgments are due to the Committee for this financial help they have given.

For the investigation of this problem, the help and co-operation of my colleagues, Mr. R. G. Allan, Principal, Agricultural College, Nagpur, Dr. W. Youngman, Economic Botanist for Cotton, and Mr. A. R. P. Aiyer, Agricultural Chemist, have been necessary and have been ungrudgingly given. My very best thanks are due to my these colleagues, whose advice has been of much value to me.

My thanks are also due to Mr. Jiwan Singh and Mr. Paranjape, temporary assistants to the Government Mycologist, for all the help they have given me in conducting the experiments and for doing conscientiously and intelligently the work assigned to them.

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CONTENTS.

COTTON WILT.

BY

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(Received for publication on 19th April, 1929.)

INTRODUCTION.

In the Central Provinces and Berar the most important diseases of cotton are the seedling blight and wilt. The former disease is not so well-known as it deserves to be, because any diseased condition of cotton seedlings or plants has been usually attributed to wilt, unless the damage caused by white ants and other insects has been too obvious. But the writer is certain that in these Provinces, in the early stages of the development of the cotton crop, it suffers chiefly and considerably from seedling blight, not due to wilt but to *Rhizoctonia*. During the first six weeks or so after the seed has germinated, seedling blight develops to such an extent, that large portions of rows in the fields are laid bare as a result of this disease, which so much reduces the stand that thinning becomes unnecessary and in some cases replanting may become essential. At this stage deaths from real wilt are negligible. This *Rhizoctonia* disease, unlike the damping off disease and sore-shin, does not produce collar rot, but is at first chiefly found on leaves and primarily on cotyledons. From the leaves it may travel to the growing point and kill the whole plant or the affected leaves may be shed and visibly no further damage to the plant may be done; but it is not improbable that the plant may be so weakened that it may become susceptible to wilt and even to infection from soil fungi, such as *Fusarium;* cases have been found where this soil fungus is present in the underground tissues of plants having their aerial parts attacked by *Rhizoctonia;* but these plants generally do not show either the external or the internal symptoms of wilt; however plants may be attacked both by *Rhizoctonia* and wilt, in which case they may show external effects of *Rhizoctonia* and internal effects of wilt. In some cases the death of seedlings has been found to be due to *Phytophthora* spp. In the seedling stage some plants have been found to be diseased and dying, but no organism has been isolated from them, though they showed typical internal and external wilt symptoms. Innumerable seedlings and plants have been microscopically examined, but in no case has *Fusarium* been found in diseased plants, which either do not show signs of wilt or which are not infected by *Rhizoctonia* or any other disease; whereas *Fusarium* has been, at times, found in healthy seedlings and plants. From time to time, numerous seedlings, growing in "wilted" soil, which to all external appearances

were perfectly normal, have been microscopically examined, and they have been found to show traces of browning in their internal tissues and a few of the vessels and cells have been found to be clogged, as in typically wilted plants. The browning is negligible and the number of cells and vessels filled with the brown substance is very small; and this may explain the reason why the seedlings showed no external signs of wilt.

Typical cases of wilt begin to appear generally after the plants are more than four weeks old and they may get diseased at various stages of their growth, right up to the end of the cotton season.

Cotton wilt is known to occur in the cotton tracts of Berar, both in heavy and light soils, and in some parts of the Central Provinces. This disease is chiefly found in fields which have been continuously under cotton for several years, in fields which are water-logged, or when cotton is grown on unsuitable soils, such as heavy clay soil (known as "tel-chikny" soil), yellow coloured silted soil ("sonawali" land), or in soil overgrown with the weed (*Cyperus rotundus*), locally known as "Nagar Motha" or "Lahi". Wilt is also common in fields manured with poudrette or unrotted farmyard manure. Fields, in the near vicinity of villages, locally called "akhar" fields, are also particularly susceptible to wilt. The incidence of wilt is not the same year after year. In some years it is very much more than in other years. Wilt has not been known to spread from field to field or from plant to plant. A wilt affected field may be surrounded by fields bearing a perfectly healthy crop of the same susceptible variety and *vice versâ*; for example, in the Agricultural College Farm at Nagpur there is a particular field which is so badly wilted that it is absolutely uneconomical to grow on it susceptible varieties of cotton, like *roseum*; but fields in its immediate neighbourhood can grow a perfectly healthy crop of *roseum*; saucer shaped parts of a field and those portions of it through which rain water naturally winds its way, for want of proper drainage, bear wilt affected plants, but a healthy crop may be borne by the rest of the field, which is properly levelled and drained. Wilt may appear in fields or portions of fields which were not known in the past to be wilt affected, but these new patches of wilt have no bearing on the presence of wilt affected fields.

Symptoms.

(1) *Macroscopic.* If the wilt affected plant bears the cotyledonous leaves, the presence of the disease in the early stages is evident by the turning yellow of these primary leaves, their green colour is generally, wholly or partially, replaced by yellow colour; they do not lose either their turgidity or their normal shape, but are shed very easily by the lightest touch or by the slightest blow of wind; both the base of the petiole and the node, from where it has dropped off, show a tiny brown central ring. The foliage leaves may show loss of turgidity and the apical succulent portions of the plant may droop and then it may ultimately die. If the plant becomes wilt affected at a little later stage, the disease is, as a rule, mani-

fested by one or two of the lower foliage leaves either turning yellow from margin inwards or by a general fading of the bright green colour which is replaced by a mixture of light green and pale yellow ; but the green colour in either case is more persistent in the neighbourhood of the main veins ; the affected leaf may also have a mottled appearance ; whether the loss of colour is general or partial, the lamina loses its turgidity and bends over at the point of its union with the petiole ; the lamina is soft to the touch and the margin of the lobes roll inwards. A little later the petiole may also lose its turgidity and droop ; other leaves may also become likewise affected and show a gradual loss of colour ; these wilt symptoms may be shown by any part of the affected plant or they may be confined only to those parts that are in the same vernation, showing that the vascular bundles of only one side of the xylem are not functioning. A little later, the whole plant may droop. At times the whole plant, all of a sudden, shows loss of turgidity, the first suspicion of the disease being a slight loss of colour of one or more of the leaves. The upper tender portion of the stem bends down on itself in a graceful curve ; the fully opened or partially opened and unopened leaves and buds also droop. There is no immediate loss of the green colour in the upper parts of the stem ; they lose their green colour rather gradually. The margins of these fully opened and partially opened green leaves roll inwards. In this condition not only the lamina is drooping but the petiole as well. The drooping leaves do not turn brittle ; but remain soft for a long time. The leaves of a wilting plant do not readily and necessarily turn brown, even if their green colour has been completely replaced by pale yellow. These leaves may drop off without turning brown. If any of these affected leaves or twigs are plucked, a brown ring in the xylem portion is visible on the exposed parts. After some time the stem of the diseased plant begins to turn brown from top downwards. Very often this browning is only on one side of the stem. The browning does not necessarily start from the apex downwards, but it may begin from an intermediate node. This browning increases elliptically chiefly along the main axis and ultimately the whole or part of the stem is girdled. The branch arising from the node may also begin to turn brown from the base upwards. The browning of the main stem may travel downwards and involve the whole plant or it may get checked and new shoots may be put forth from the lower nodes. These shoots may produce flowers and bolls as well. The leaves of badly wilted plants are shed and plants with bare branches and twigs are left standing in the fields. If the plant is not completely dead, it is not unusual to find the dormant axillary buds, especially those near the base of the stem, sprouting.

The underground parts of a wilting plant may or may not show any outward visible signs of wilt. In some plants the collar is distinctly swollen, as a rule, elliptically to a length of about an inch or less, and this swollen part has one or more vertical cracks which are at first white ; but in a majority of cases the underground portions are normal. If a wilted plant is left long in the soil, its underground parts turn wholly brown or black and rot ; and their bark is in shreds. This rotting

is not directly due to wilt, but seems to be the result of the action of saprophytes, such as bacteria, eelworms and various fungi.

If the bark of a wilted or wilting plant is removed, the white wood is found to have longitudinal brown streaks, running from the roots right up to the apex and also to the branches. In wilted plants which have been allowed to stand in fields for a long time, these brown streaks may not be so marked and they may also lose their individuality as the wood becomes wholly brown or black.

(2) *Microscopic.* In transverse sections the wilt symptoms are very typical. In the early stages of wilt, when the plant hardly shows any external symptoms, the presence of the disease is evident by the walls of some of the cells and vessels of the xylem tissues being discoloured yellow. At this stage the cells and vessels are not filled with any brown or yellow substance and there is no trace of fungus hyphæ in the tissues; in advanced cases the walls of many of the vessels and cells of the xylem tissues are discoloured yellowish brown or brown or black and their lumen may be completely or partially filled with a black or a dark brown coloured solid substance (Plate VI, figs. 2 and 11). In some of the cells of the xylem tissues are developed tyloses (Plate VI, fig. 5). There is often a lot of starch formation in these tissues and in the medullary rays (Plate VI, fig. 6). The plugging of the vessels is particularly more so in the nodes of the stem and at the points where rootlets arise (Plate VI, figs. 9 and 10). The discolouration of the vessels explains the cause of the longitudinal brown streaks that are found on the wood underneath the bark. The discoloured vessels filled with the dark coloured substance do not necessarily have hyphæ in them. In fact, more often than not, hyphæ are found in otherwise normal cells and vessels and their lumen are hardly completely filled by them (Plate VI, fig. 12). The amount of hyphæ present is so small as not likely to interfere appreciably with the normal functions of the affected tissues. These small quantities of hyphæ have also been found by Ajrekar and Bal,[1] Rosen,[2] Fahmy [3] and others. The browning in a wilted plant is found throughout the plant, but the hyphæ are not necessarily found in all parts of the plant; for example, in the apical regions and side branches there are very often no hyphæ, though the browning is very conspicuous. In the subterranean parts as well, though the browning may extend right up to the tips of the tap root and laterals, still fungus hyphæ may not necessarily be found in all these browned parts. The tissues of a wilting plant are very much similar to those of a mature, evidently healthy plant at the end of the season, in February or March, growing in a wilt infected soil. A large majority of old plants, to all appearances normal, growing in a "sick" soil, show

[1] Ajreker, S. L., and Bal, D. V. Observations on the wilt disease of cotton in the Central Provinces. *Agri. Jour. India*, Vol. XVI, pp. 598—617, 1921.

[2] Rosen, H. R. Efforts to determine the means by which the Cotton-wilt fungus, *Fusarium vasinfectum*, induces wilting. *Jour. Agri. Res.*, XXXIII, pp. 1143—1162, 1926.

[3] Fahmy, T. The *Fusarium* disease (wilt) of cotton and its control. *Phytopath.*, XVII, pp. 749—767, 1927.

a certain amount of browning of the vessels, a large amount of starch formation and tyloses and at times also *Fusarium* hyphæ (Plate VI, figs, 3, 7 and 13). The only difference between the mature and old plants and wilting plants being that in the latter, the browning is much more than in the former. Evidently the wilting plant is suffering from premature senility.

(3) *Microchemical.* The microchemical reactions of a wilt affected plant are very conspicuous and they are as typical as the morphological and histological symptoms. Sections of wilted plants treated with ammonium carbonate and log-wood have invariably stained blue the discoloured vessels and cells. The healthy parts of the tissues remain unstained.

Different varieties of Alizarin and Brasilin stain the diseased parts of the plants beautifully bright red. In the infected tissues of the wilted plant, it is the cells and vessels of the xylem that stain red or bright pink. The medullary rays remain unstained. Those cells of the xylem that may contain hyphæ but are not discoloured or plugged with the brown substance remain unstained.

Some seedlings, growing in " sick " soil. though externally perfectly normal and healthy, have shown slight browning in the internal tissues of their underground parts and a few cells and vessels have been found to be plugged as in typically wilted plants. These affected parts of the tissues have given the same microchemical reactions as the affected tissues of wilted plants. These plants have been found to be quite free from fungus infection.

Plants to all appearances perfectly normal growing in " sick " soil, at the end of the cotton season, give more or less the same microchemical reactions in the discoloured cells and vessels as those of wilted plants ; but the plugging and discolouration of the vessels is very much less than in wilted plants, not enough to interfere materially with their normal activities. The normal protoplasmic contents of many of the cells in the cortex of these plants are also stained red. Not only do the cortical cells of old healthy plants growing in " sick " soil give these microchemical reactions but healthy plants growing in non-wilted soil give the same reactions in the cortical cells at the end of the season. These reactions are not peculiar only to susceptible varieties, like *roseum*, but even wilt resistant varieties, like Buri, behave similarly. On the tissues of young healthy plants, Alizarin is found to have no effect. This shows that in the cell contents of the old plants there is something which is not found in the young plants and which stains blue with ammonium carbonate and log-wood, and red with Alizarin or Brasilin. This " something " is also found in wilting plants, but in the xylem tissues instead of in the cortical cells, and it completely or partially blocks the lumen of many of the xylem vessels and cells. Therefore this " something " must have been absorbed from the soil ; in wilting plants it gets aggregated or precipitated in the xylem tissues, whereas in healthy plants it remains mobile and is translocated from root upwards. Thus it appears once again that wilt is a sort of premature senility.

COMPARISON WITH COTTON WILT OF OTHER COUNTRIES.

Cotton wilt is known in many parts of cotton growing tracts of the world. The incidence of this disease was first reported from America in 1892 by Atkinson.[1] He attributed the cause to *Fusarium vasinfectum*. In India it has long been known in the Central Provinces and Berar, and Bombay, the first mention of cotton wilt being by Evans [2] in 1908, and recently it has been reported from Burma [3] as well. Cotton has been known to suffer from wilt also in Argentina, South Africa, Egypt, Dardanelles and other countries where cotton is grown, but as far as the writer is aware, the morphological and histological characteristics of the wilt of cotton occurring in different cotton-growing countries have not been carefully described, except by Atkinson and by Neal [4] in America, and by Fahmy [5] in Egypt, who considers the Egyptian wilt to be different from the American and the Indian wilts. All that we so far know is that either *Fusarium vasinfectum* or *Fusarium* sp. has been obtained from wilted plants. The mere presence of *Fusarium vasinfectum* or of *Fusarium* sp. in wilted plants does not necessarily signify that the plant is suffering from wilt or that the cotton wilts of different countries are identical.

If our description of cotton wilt be compared with the descriptions of the American cotton wilt, given by Atkinson and by Neal, some important differences are readily noticeable.

According to Atkinson, the leaves of the diseased plants have "three distinct colours, green, yellow and brown, in parallel radiating bands. The brown and dead parts of the leaf soon break out, leaving the leaf quite ragged." These tri-coloured parallel radiating bands are not characteristic of the wilt under study, and secondly, the discoloured leaves do not become ragged by the diseased parts breaking away from the healthy parts. Atkinson has found the discolouration of the tissues "more produced in those ducts in which the fungus is located," whereas we have already seen that the fungus, if present, is, more often than not, found in cells which are normal and not discoloured.

Neal's recent account of the American wilt is not very descriptive, but still it is sufficient to bring out the points of differences between the two wilts. He finds "a noticeable yellowing of the leaves of the cotton plant accompanied by a stunted appearance, somewhat early in the season, is usually a good indication of the *Fusarium* wilt"; the tap-root of a diseased plant is also found to be stunted. Cotton plants develop wilt in these Provinces at all stages of their growth, but neither their stems nor their tap-roots have been found to be stunted (Pl te II, Figs. 1 and 2). Neal has further observed that the "disease may be in irregular spots

[1] Atkinson, G. F. Some diseases of cotton. *Ala. Agri. Expt. Stn. Bul.* 41, pp. 19—29, 1892.
[2] Evans, G. Cotton wilt in the Central Provinces. *Agri. Jour. India*, Vol. III, pp. 78—80, 1908.
[3] Rhind, D. Report of the Mycologist, Burma, for the period ending 30th June, 1924—Rangoon, Superintendent Government Printing and Stationery, Burma, 1924.
[4] Neal, D. C. Cotton wilt: a pathological and physiological investigation. *Ann. Miss. Bot. Gard.*, XIV, pp. 359—424. 1927.
[5] Fahmy, T. The *Fusarium* disease of cotton (wilt) and its control. *Ministry of Agri. Egypt., Tech. and Sci. Service, Bull.* No. 74, pp. 1—106, 1928.

over the field and each succeeding year the infected areas enlarge." We have found the wilt to occur in spots over a field, but the infected areas have not been found to be enlarging each succeeding year.

The detailed description of the Egyptian wilt, given by Fahmy,[1] brings out some important pointsof differences between the wilts of cotton in Egypt and in these Provinces. In Egypt, in severe cases of wilt, the affected cotyledons, wholly or partially, dry up; diseased plants, which have recovered, show marked dwarfness; infected plants, in which the dying of the stem from apex downwards is checked, develop abnormal branching; the dead portions of a wilted plant turn nearly black and on drying become somewhat curved; "infected plants whether they show external symptoms or not, are generally dwarfed"; the discolouration of the internal tissues is not so characteristic in the infected seedlings as in the case of old plants. These are some of the important symptoms which are different from those of the cotton wilt under study. The infected cotyledons turn wholly yellow and may be shed without drying up; affected plants, when the progress of the disease has been checked, do not become dwarfed (Plate II, figs. 1 and 2); the drying stem may turn dark brown or almost black, but on drying, it has not been found to become curved; the internal discolouration, which is brown in colour, is the only unfailing evidence of wilt infection in a cotton plant or seedling, whatever be its age. Fahmy has compared "the Egyptian, Indian, and American *Fusarium* parasites, producing the wilt disease in the respective countries," and has found them to be different in certain characters.

It therefore seems that the cotton wilt in these Provinces is different from the disease known as wilt in Egypt and America.

Kulkarni's[2] account of the symptoms of the cotton wilt disease in the Bombay Karnatak can apply equally well to the disease in these Provinces as to wilt in other countries.

Pot Culture Experiments.

For these experiments, unless otherwise mentioned, a variety of *Verum* type of cotton, *viz.* AK2, was used, as it is highly susceptible to wilt. This cotton was very kindly supplied by Dr. Youngman, Economic Botanist for Cotton.

Unless otherwise stated, cotton was sown, each year, at the normal time, *i.e.*, soon after the break of the rains.

The *Fusaria* isolated from cotton soils and plants, referred to in this paper, are identical with those described by Jiwan Singh.[3] The nomenclature used by him for distinguishing these *Fusaria* has been retained, as their taxonomic positions have not been as yet established.

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[1] Fahmy, T. Loc. cit.

[2] Kulkarni, G. S. II. The parasitism of the *Fusarium* associated with the Wilt Disease of Cotton. *Mem. Dept. Agri. India, Bot. Ser.*, XVII, pp. 11—20, 1928.

[3] Jiwan Singh. A study of *Fusaria* common to Cotton Plants and Cotton Soils in the Central Provinces. *Mem. Dept. Agri. India, Bot. Ser.*, XIV, pp. 189—198, 1927.

Manures. Lime was applied to wilt affected soil in varying proportions, but it had no effect on the control of wilt and there was no definite relationship between the incidence of wilt and the quantity of lime applied.

Superphosphate did not influence the incidence of wilt the year it was applied, though the growth of individual plants was decidedly better than of those that did not receive this manure. The stem was thicker and longer and produced more branches, the leaves were greener and the number of bolls per plant was larger; but there seemed to be some residual effect of this manure on wilt and it was most marked in those cases where a large quantity was used. In pots containing "wilted" soil to which was given super, in 1924, at the rate of 5 tons per acre, there was 50 per cent. of wilt; in 1925, when these pots were replanted without any further treatment, there was no wilt, and in 1926, the wilt was 12 per cent. In 1927, 16 per cent. of plants became wilted; those pots, which had received in 1924 super at the rate of 1 ton per acre, gave 75 per cent. of wilt in 1924, 25 per cent. in 1925, 33 per cent. in 1926 and 25 per cent. in 1927. Lesser quantities of super did not seem to have any residual effect.

When super was given with lime, there was no residual effect. Lime alone also in no way showed any residual effect.

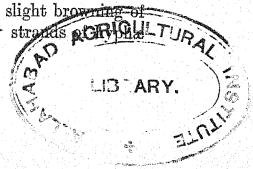
In 1927, lime was added to healthy soil, at the rate of 10 tons to the acre. Wilt to the extent of 9 per cent. was developed in this limed soil, whereas in the control healthy soil there was no wilt. In 1928, this limed soil without any further treatment was resown with *roseum*; the amount of wilt was much higher than in the previous year. In 1928 *roseum*, sown in healthy soil, which was treated with lime at the same rate as in the experiment of the previous year, became badly wilted, whereas *roseum* in the untreated healthy soil remained healthy.

In 1924 "non-wilted" soil was treated with heavy doses of manure such as farmyard manure, rotted castor cake and rotted *nim* (*Melia Azadirachta*) cake. Some plants growing in this highly manured "non-wilted" soil showed typical signs of wilt. In 1925 these experiments were repeated; some plants began to wilt by the end of July. Some of the pots in which all theplants were healthy were inoculated on the 24th of August with *Fusarium* Strain A, commonly found in wilted plants. The inoculation was done by pushing an iron rod into the soil at different depths varying from 6 inches to 3 feet and filling the holes thus made with water containing a culture of the fungus. At the end of the season it was found that in the uninoculated manured soil, out of 36 plants of the susceptible variety AK2, 5 plants had died of wilt and 6 of *Rhizoctonia* and out of 36 plants of a partially resistant variety, a *Malvensis*, AK4, 3 had died of wilt and one of *Rhizoctonia*; and in the inoculated soil out of 36 plants of AK2, 2 had died of wilt and 5 of *Rhizoctonia*; and out of 36 plants of AK4, 3 had died of wilt and one of *Rhizoctonia*. In these pots the following year, *i.e.* in 1926, AK2 was again sown, plants began to wilt from the end of July. Those inoculated pots of the previous

year, which had not as yet given any wilted plants were, at the end of August, reinoculated, similarly as in 1925. The percentage of wilt was higher in 1926 in these series, but was more or less the same in the inoculated and uninoculated pots.

In 1926 plants were again grown in a highly manured " non-wilted " soil and the same results were again obtained, wilt appeared both in the inoculated and uninoculated pots and the amount of wilt in them was more or less the same.

Another manurial experiment that deserves special mention is the one in which was used *nim* cake. In 1925, this cake manure was used with castor cake. Since all the plants growing in this series were healthy and showed very good development, 12 plants were sectioned and examined microchemically between December 1925 and January 1926. Of these 12 plants, one showed a little browning of a few of the vessels, and the lumen of some of the xylem cells were plugged with the brown substance ; this plant gave with Alizarin the typical bright red colour only in the discoloured parts of the xylem tissues, but in its cortical cells the living protoplasm stained typically red. The remaining 11 plants also gave this peculiar reaction only in the cortical cells. There was no plugging of the cell lumen, and the unstained sections looked perfectly normal. But the remaining plants examined a few weeks later, at the end of February of 1926, were very characteristic ; to all external appearances the plants were normal but when they were uprooted, everyone of these 28 plants had on its roots galls of varying sizes—galls similar to those found on cotton roots attacked by nematodes (Plate V, fig. 2). However, no nematodes were found in these galls. The tissues of these galls showed no plugging, but with Alizarin there was a diffused bright red colouring of the protoplasm ; there was no browning in these galls nor were there any hyphæ ; only one plant showed a little browning and that too in the normal part of the tap root, a little above the gall ; though in the galls themselves there were no hyphæ, but in all these plants hyphæ were found in the tap roots, above the galls ; in cultures, from every one of these tap roots was obtained the *Fusarium*, generally found from wilted plants. Thus though this *Fusarium*, *viz.* Strain A, was found in these plants, still they were perfectly healthy and normal, except for the galls. Cotton sown in 1926 in these pots of 1925 without any further treatment was wilted to the extent of 60 per cent. In 1926, the " non-wilted " soil from the Nagpur College Farm was treated with farmyard manure and *nim* cake. In this series 23 per cent. of plants were typically wilted. In February 1927, there were 11 plants in the pots containing soil manured in 1925 with *nim* cake, and 25 plants in pots having the soil similarly manured in 1926. All these plants looked perfectly normal. Five plants from each of the two series again had on them root galls similar to those described above. In transverse sections of the galls stained with Alizarin, the protoplasm was coloured uniformly bright red but there was no plugging of the cell lumen. There was neither any browning of the gall tissues, nor did they have any fungus hyphæ. In some cases there was a slight browning of the xylem tissues of the normal root above the galls, and a few strands of



were found in them. The fungus, when cultivated, proved to be *Fusarium* Strain A. Similar results were obtained the following season.

Fungicides. In 1925, wet and dry Uspulun were tried to see the effect of these fungicides on wilt. Dry Uspulun was mixed with wilted soil at the rate of 17 cwt. per acre. The growth of plants was excellent. The plants were standing at least a couple of feet higher than those growing in the fields or in pots and were at least three times bigger than those growing in wilted soil. The average number of bolls per plant was 9·75, whereas in the untreated wilted soil it was 4·2 and in the untreated healthy soil 9·3. Though in 1925, in fields and in pots, there was a very heavy attack of *Rhizoctonia* which killed many plants, still in this Uspulun treated soil there was not a single case of *Rhizoctonia* infection. Wilt in these pots was very little. The beneficial effect of this treatment was persistent even in the following years. As this fungicide is very expensive, the cost of applying it at the rate given above would be prohibitive. Therefore varying smaller quantities were tried in 1926, but it had no effect in controlling wilt in these small quantities though the growth of the plants was better than of those in the untreated soil.

Wet Uspulun gave practically the same results as dry Uspulun, *i.e.* heavy doses of this fungicide controlled wilt but at a prohibitive cost, whereas smaller doses had no effect on wilt, though they controlled *Rhizoctonia*.

Pickling of seed with Uspulun solution had no effect either on the growth or the disease resistance of the crop.

"Wilted" and "Non-wilted" soils. In 1923, some experiments were conducted to see the effect on plants growing in pots containing both "wilted" and "non-wilted" soil. In one series the lower half was filled with "wilted" soil and the upper half contained "non-wilted" soil obtained from a tank near Nagpur; in a second series, the relative positions of the two soils were reversed, *i.e.* in the lower half there was "non-wilted" soil and the upper half had "wilted" soil. Though both these series were exactly alike, except for the relative positions of the two soils in the upper and lower halves of the pots, the results were not identical. Wilting of plants was first noticed in those pots which contained "wilted" soil in the upper half, whereas in the other series wilt appeared much later; but still at the end of the season there was not a single plant alive in this series; whereas in the former case, even though wilt had appeared earlier, at the end of the season, there were some healthy plants. A few plants from these two experimental series were carefully removed to examine their roots; from the condition of the roots it could be easily made out whether their upper or lower parts were growing in the "wilted" or "non-wilted" soil. Those portions of the roots which were growing in the "wilted" half showed more browning than those growing in the healthy half of the same pots and a larger percentage of the fine roots was dead in the "wilted" portion than in the "non-wilted" portion. These experiments repeated in 1924, 1925, 1926 and 1927 have given similar results. Pots containing "wilted" soil in the upper half have

always had the incidence of wilt earlier, but a larger number of surviving healthy plants at the end of the season than pots which had healthy soil in the upper half.

A modification of these experiments was tried in 1925.

Six inch earthen pots having extra large drain holes, at the bottom, were filled either with healthy soil or " wilted " soil ; each of these was embedded up to the brim in a three foot high glazed pot containing either wilt infected or healthy soil, according as the small pot had healthy or wilt infected soil, *i.e.*, the small pot with " wilted " soil was placed in the big pot with healthy soil and the one with healthy soil was embedded in " wilted " soil. Cotton seeds were sown both in the small and in the big pots after the former had been placed in their proper position. There was one other parallel series, but with this difference that the seeds were sown both in the big and small pots, but the latter were not embedded in the former till the seeds had germinated and the roots had come out through the drain holes of these small pots. In both the series the results were the same. In the big pots containing " wilted " soil most of the plants were wilt infected, whereas there was not a single case of wilt in those big pots which had healthy soil ; but plants in the small pots containing healthy soil began to wilt after their roots had penetrated the " wilted " soil in the big pots ; but of the plants in the small pots with " wilted " soil, a few wilted, and they became diseased when they were growing wholly in the " wilted " soil ; but when the remaining healthy plants put forth their roots in the healthy soil of the bigger pots, there were no more cases of wilt.

In 1925, cotton was grown in soil containing different proportions of " wilted " and " non-wilted " soils and it was found that higher the proportion of " wilted " soil to the " non-wilted " soil, higher was the percentage of wilt ; but the following year, in these mixed soils, wilt was more or less the same ; the plants in pots containing a major part of " non-wilted " soil was as badly wilted as those grown in the same quantity of " wilted " soil. In 1926, this experiment was repeated and it was again found that the percentage of wilt was higher, greater the quantity of " wilted " soil in the mixture of " wilted " and " non-wilted " soils.

Chemicals. In " non-wilted " soil, different proportions of aluminium nitrate and aluminium sulphate were added before sowing seeds of AK2. 0·2 per cent. and 0·1 per cent. of the nitrate salt each gave 6 per cent. of wilted plants in 1925, whereas 0·05 per cent. of this salt gave no wilt, and 0·1 per cent. of the sulphate salt gave only 3 per cent. of wilt. These diseased plants showed typical, macroscopic, microscopic and microchemical characteristics of wilt, and *Fusarium* Strain A was isolated from them.

Some of the healthy plants were microchemically examined in November 1925. Sections of plants growing in the soil containing 0·2 per cent. of the sulphate showed with Alizarin a diffusion of bright red colour in the cortical cells ; but plants growing in soil containing 0·05 per cent. of the nitrate hardly showed any reaction with the stains in the cortical cells. No browning was found in the tissues of the healthy plants.

Buri, a totally resistant variety, was also grown in soils containing the same proportions of the sulphate and nitrate salts as in the above experiments. The plants were perfectly healthy and showed very little of the pink colour in the cortical cells when the sections were stained with Alizarin. The soils treated in 1925 were resown with AK2 the following year. Plants growing in soils containing 0·2 per cent. and 0·1 per cent. of the nitrate were wilted to the extent of 15 per cent. and 21 per cent. respectively; whereas those growing in soils containing 0·05 per cent. aluminium nitrate and 0·1 per cent. aluminium sulphate remained healthy.

In 1926 a fresh lot of healthy soil was again treated with 0·2 per cent., 0·1 per cent. and 0·05 per cent. of aluminium nitrate or aluminium sulphate. The results of 1926 were not similar to those of 1925. The percentage of wilt in soil containing 0·2 per cent., 0·1 per cent. and 0·05 per cent. of the nitrate salt was 12, 4 and 12 respectively, whereas in the case of the soil similarly treated with the sulphate salt, the percentage was 66, 16 and 16 respectively. To another lot of soil similarly treated with these various proportions of these two salts were added cultures of *Fusarium* Strain A, before sowing the seeds. A third lot of the aluminium treated soils was inoculated with this fungus two and a half months after the seeds had been sown. In the soils treated with 0·2 per cent., 0·1 per cent. and 0·05 per cent. of aluminium nitrate and inoculated with the fungus before sowing the seed, the percentages of wilt were 75 and 62 respectively, whereas in treated soil inoculated after sowing the seeds the percentage of wilt was 16, 20 and 25 respectively. In the soils treated with 0·2 per cent., 0·1 per cent. and 0·05 per cent. of the sulphate salt and inoculated before sowing the seed, the percentages of wilt were 58, 66 and 62 respectively and when the fungus was added in September after the seed was sown in July, the percentages of wilt were 42, 4 and *nil* respectively.

Buri plants grown in soils containing even higher proportions of aluminium salts and in similarly treated soils to which the fungus was added, either before sowing the seed or two months after sowing the seed, remained healthy. There was no browning of the tissues of any of these plants, but when sections were treated with Alizarin, they showed a diffused pink colour in the cortical cells and that too only of those plants growing in soils treated with the higher proportions of the salts.

To healthy soil was added pure nitric or sulphuric acid in proportions to give the same number of NO 3 and SO4 ions as in 0·2, 0·1 and 0·5 per cent. of the nitrate and sulphate salts of aluminium. These acids were diluted with distilled water before adding them to the soil. In the pots containing the highest amount of nitric acid 6 out of 24 plants became wilted. In each of the remaining two proportions, 2 plants out of 24 wilted. When the SO4 ions were added, no plants wilted except in the soil containing the least quantity of the ions, 3 plants having wilted out of 24.

In 1927, 70 c. c. of 4N sulphuric acid was added to 60 lb. of "wilted" soil; 4 per cent. of the plants became wilted, whereas in the control pots containing untreated "wilted" soil, 29 per cent. of the plants died of wilt; but when the quantity of the acid was doubled, the percentage of wilt increased from 4 to 12. These pots were

sown with *roseum* in 1928 without any further treatment ; wilt was less in the pots containing the smaller dose of acid than in the pots containing the larger quantity.

Sterilization of soil. In 1925, " wilted " soil was dry sterilized at 150°C. for one hour, and was filled in sterlized 9-inch pots. They were sown with AK2 cotton. The growth of the plants was very poor, but there were no cases of wilt, whereas 20 per cent. of the control plants, growing in the unsterilized " wilted" soil, died of wilt. These pots, with sterilized " wilted " soil, were resown the following year and the plants growing in these pots were once again free from wilt.

In 1926 and 1927, fresh lots of " wilted " soil were dry sterilized at 120°C. for two hours ; sterilized 9-inch pots were filled with these soils and planted with AK2 cotton. A few plants developed wilt in these series.

In 1928, this experiment was again repeated ; " wilted " soil, sterilized at 120°C. for two hours, sown with *roseum*, produced a few wilt affected plants ; but *roseum* grown in " wilted " soil, sterilized at 140°C. for two hours, was not wilt affected. The growth of plants in these sterilized soils was again very poor.

Effect of jowar (Sorghum) on the incidence of wilt. In 1926, 12 pots filled with wilted soil were first sown with *jowar* on the 24th of June and a week later some of them were sown with AK2 and the rest in the end of August, when the *jowar* plants were well grown. Very few plants died of wilt in these series, though in the control pots the percentage of wilt was 59.

In 1927 and 1928 *jowar* and cotton were again similarly grown together in " wilted " soil ; and the incidence of wilt was again found to be negligible.

Though the amount of wilt was so little in pots in which cotton and *jowar* were grown together, still the cotton plants were very poor in growth.

Injection experiments. In 1923, experiments were conducted to see the effect of injecting, in plants, solutions of different strengths of aluminium and iron salts. The results have already been recorded in a previous publication.[1] In 1925, plants were again injected with (1) N/300 solution of pure aluminium nitrate, (2) a similar solution mixed with spores of *Fusarium* Strain A, (3) distilled water, and (4) distilled water containing spores of *Fusarium* Strain A. AK2 and AK4 varieties were used for these experiments. The injection was made by means of a glass tube bent at right angles and having a fine point drawn out at the end of the bend. The finely drawn out end was pushed into the stem through a puncture made by a sterile needle at soil level or a little lower. The joint between the glass tube and the stem was made water tight by means of plastecine. The tube was filled with the liquid to be injected.

Fo. these injections 3 months' old plants having a well developed thick stem were selected ; a month and a half after they were injected, the plants were microscopically examined. Those plants which were injected with distilled water or distilled water and fungus spores showed no browning of the inner tissues—there was a slight brown-

[1] Dastur, J. F. A Preliminary Account of the Investigation of Cotton Wilt in the Central Provinces and Berar. *Agri. Jour. India*, XXIX, pp. 251—260, 1924.

ing immediately round the wounded parts and sections of the stem at the place of injection gave no reaction with Alizarin. But plants injected with aluminium nitrate solution with or without spores showed typical browning of the tissues half an inch below and above the puncture through which the solution was injected. In the xylem cells there was the typical clogging of the vessels and cells situated near the side of the stem where the injection was given. With Alizarin, the sections from the portions which had absorbed the solution gave the typical pink reaction as in wilted plants. In the plants injected with the salt solution containing fungus spores there were no hyphæ; the portions of these stems, where the injections were done, were externally sterilized and incubated in sterile tubes containing culture media. These pieces remained sterile.

A few days after the injection experiments were started, there was a slight yellowing and partial drooping of some of the leaves of plants, injected with aluminium solutions. These leaves were situated on the side of the stem where the injecting tube was introduced. This yellowing and drooping was only temporary, the leaves regained their normal colour and position in a day or so.

This method of injecting liquids in plants was found very unsatisfactory, because during the months these experiments had to be done, there were generally strong winds blowing, at times accompanied with a heavy rain-fall and therefore the injecting tube very often either broke at the point where it was introduced in the stem or got displaced. Therefore in 1926, the injection experiments were carried out in a different way which proved to be quite satisfactory. The liquid to be injected was filled either in a calcium-chloride tower or in a burette, which was placed on a higher level than that of the plant to be injected. The lower opening of the apparatus was connected with the part of the plant to be injected by means of a piece of rubber tubing. The liquid was injected through the cut end either of a leaf petiole or a small twig or a lateral root, the diameter of the rubber tubing being the same as that of these plant organs, which were cut under water; the free end of the rubber tubing was then slipped over them, the liquid to be injected was allowed to flow out when the rubber tubing was being connected with the plant so that between the liquid and the cut end of the plant part there could be no air bubbles. This method of injection was found to be very satisfactory, first because the liquid was constantly in contact with the plant and secondly because the amount of liquid absorbed could be recorded.

Plants of AK2 were injected with 1 per cent. and 0·5 per cent. of aluminium nitrate solutions through cut ends of petioles. The liquid was gradually absorbed and on the third day after the experiment was started, the leaf petioles showed distinct signs of browning and looked pale and sickly. Leaves and leaf buds, in the same vernation as the injected petiole, were drooping as in the case of typical wilt.

The node bearing the injected petiole showed externally the peculiar browning, and which increased elliptically, as in the case of a normally wilted plant. Similar browning was also found at the nodes of the leaves in the same vernation. The

leaves lost their flaccidity, became limpid and drooped. The lamina marginally rolled inwards and there was also the yellowing of the leaves ; they showed all the signs of typical wilt (Plate I, fig. 2).

The stem of plants injected with 0·5 per cent. aluminium nitrate, when cut open, showed typical browning at the nodes ; the internodes did not show this browning. In about 4 or 5 days after injection, the plants showed characteristic signs of wilt. In sections made through the nodes there were a number of discoloured vessels and cells of the xylem tissues, the lumen of which were typically plugged as in naturally wilted plants. The tissues of the internodes looked normal ; when sections through the nodes were treated with Alizarin or Brazilin, they stained exactly like the sections from a wilted plant ; sections from the internodes, when similarly stained, showed a diffused pink reaction in the protoplasm of the tissues.

Plants injected with 1 per cent. solution of the aluminium salt died as if they were attacked by wilt and they showed all the macroscopic, microscopic and microchemical symptoms of real wilt (Plate VI, figs. 4 and 8).

Fungus hyphæ were not found in injected plants at first, but when they were left in pots for a week or more after they began to show signs of wilt, *Fusarium* hyphæ were found in their tissues and in cultures the fungus proved to be the *Fusarium* commonly found in wilted plants, *viz.*, Strain A.

When the injection was made with a solution containing 0·25 per cent. aluminium nitrate, the plants showed no external signs of wilt ; but there was a slight amount of browning and clogging of the cells and vessels at the nodes ; a faint reaction was obtained with Alizarin in sections through nodes.

Plants similarly injected with distilled water also absorbed the liquid ; they remained healthy. Plants similarly injected with distilled water or aluminium nitrate solutions, to each of which was added a heavy suspension of spores of *Fusarium* Strain A did not absorb any of the liquid and remained perfectly healthy. It was found that the spores formed a slimy layer on the cut end of the petiole which interfered with the absorption of the liquid. But when to the water and the salt solutions only small quantities of the spores were added, the liquids were absorbed and the results were the same as in the case of liquids injected without the addition of the spores. The petioles through which the spore-laden liquids were injected showed no trace of the presence of the fungus.

Plants injected through cut ends of twigs gave exactly the same results as those injected through the cut end of a petiole.

As in the case of naturally wilt-affected plants, the injected plants also may recover after showing the toxic effects of the injection ; new shoots may be put forth and the plants may continue their normal growth after showing the first signs of wilt. Some plants which had thus recovered were examined a month after they were injected ; some of the xylem tissues showed typical symptoms of wilt and what was most interesting was that *Fusarium* Strain A was found in abundance ; but still

the plants looked healthy ; they were able to carry on their functions because only a few of the vessels were discoloured and filled with the brown substance.

A wilt resistant variety, Buri, was similarly injected with 2 per cent. and 1 per cent. solutions of aluminium nitrate ; it showed the same internal and external signs of wilt as the injected AK2 plants ; but Buri plants injected with 0·5 per cent. solution, though they became diseased and were practically dead five days after the injection, showed more signs of drying up than of wilting. Browning of the tissues was seen near the node bearing the injected petiole and they gave the typical reaction with Alizarin.

When the injection of the aluminium salt was done through roots, the plants developed typical wilt. Some of the plants had absorbed about 15 c.c. of the 1 per cent. solution in about 11 days' time. Plants injected through roots showed the external signs of injection later than those injected through petiole or twigs. Within a week after the injection, the leaves began to droop and to lose their green colour ; and the whole plant looked unhealthy. The leaves which were pale yellow when removed from the stem showed typical browning at the base of the stalk, as in a wilted plant, and sections treated with Alizarin gave the typical pink reaction. The injected root was in every way similar to the root of a wilted plant. The browning extended to the tap root as well. The vessels showed the characteristic plugging and gave pink reaction with Alizarin. The stem showed external browning at the nodes as in a wilted plant. The injected plant not only showed the browning of the tissues and plugging of the lumen of the xylem tissues but they also showed the presence of tyloses.

The various strengths of the nitrate salt used for these root injections were 1 per cent., 0·5 per cent., and 0·25 per cent. and they all gave more or less the same results Plants similarly injected with distilled water remained healthy.

In September 1927, AK2 and AK4 and Buri plants were again similarly injected with one per cent. Al No. 3 solution and gave identical results.

Plants injected with water or water containing suspension of spores of *Fusarium* Strain A remained healthy and normal.

In 1928, *roseum* plants were injected with 0·1 per cent., 0·2 per cent., 0·3 per cent., 0·4 per cent. and 0·5 per cent. aluminium nitrate. The external signs of wilt were evident in the plants injected with the salt solutions of 0·3 per cent. and of higher concentrations. Plants injected with the weaker solutions looked externally healthy.

The injection experiments show that the most susceptible varieties like *roseum* and AK2 are more readily liable to aluminium toxicity than the more resistant variety, AK4, and the most resistant variety, Buri. Of the two, *roseum* and AK2, the former is more susceptible than the latter and also more readily shows signs of wilt, when injected with aluminium salts. AK4 is partially resistant and can stand a higher concentration of aluminium salts than the susceptible varieties. Buri, which is wholly resistant, can absorb a solution of higher concentration, and

there may be preliminary drooping of the leaves and the wood may become discoloured but unless the concentration is very high, *e.g.*, 2 per cent., there is no clogging of the cells and vessels ; the presence of the salt solution in the cell protoplasm is detected when sections are stained with Alizarin.

Last season, filtrates of *Fusarium* Strain A, cultivated on Richard's solution, were injected in the manner described above in AK2 and AK4 and Buri plants. The stale products were absorbed by the plants, but none of them showed any signs of wilt or any ill effects. Similar results were obtained when sterilized Richard's solution was used instead of the filtrates.

Transplantation of diseased plants. In 1927, some wilt affected cotton plants in different stages of disease were transplanted to healthy soil, those plants that were only slightly affected revived and put forth new growth, whereas the others completely wilted. About three months after the plants were transplanted, one of the surviving and healthy plants was carefully uprooted and microscopically and microchemically examined. At the end of the thin laterals, a few tiny burrs or root-knots were found ; these were similar to those found on plants growing in soil manured with *nim* cake ; these lateral roots did not show any browning of their tissues or the presence of hyphæ. The browning was confined only to a few old vessels of the tap root and some of these were typically plugged but the new vessels and xylem tissues, especially those near the cambium, were perfectly normal. Hyphæ also were found only in the old xylem tissues. The browning extended up to the second or third internode of the stem. The remaining upper parts of the stem were normal. The microchemical reactions of the browned parts of the tissues were similar to those of typically wilted plants. In cultures, from the collar portions of the stem, *Fusarium* Strain A was obtained.

In 1928, this experiment was repeated. *Roseum* seed was sown in soil known to be badly wilt affected. As the young plants began to show symptoms of wilt, they were carefully removed from the soil without causing much damage to the roots, which were washed to remove all the adhering soil, and then transplanted to healthy soil. It was once again found that only those of the affected plants, which were in the very early stage of wilt attack, if transplanted to healthy soil, developed into healthy plants ; plants showing, in one of the leaves, slight loss of colour were selected ; this leaf was plucked and examined ; if there were any signs of browning at the cut end, the plant was considered to be wilt affected. The surviving plants at the end of the experiment were microscopically and microchemically examined, and for the purpose of this experiment, only those plants were considered in which there was browning of the xylem tissues. This browning was confined only to a few cells of the early developed xylem tissues and they gave the typical red colour when stained with Alizarin ; the rest of the tissues remained unstained. This was a positive proof to show that the plants, when transplanted, were affected by wilt. From these surviving mature plants, *Fusarium* Strain A was isolated when a piece of the tap root near the soil level was incubated.

Inoculation Experiments. In 1925, twenty-four plants were grown in pots filled with sand to which was added a handful of " wilted " soil to serve as a sort of inoculum ; none of these plants became wilt affected.

Cotton seeds were sown in small pots filled with sand. These pots were daily irrigated through their drain holes with water containing a heavy suspension of spores of the cotton wilt fungus. None of these plants showed any signs of wilt, or any internal browning or the presence of the fungus in its tissues though the plants were poor in growth and died of starvation after they were a few months old.

In 1925, " non-wilted " soil was well mixed with small pieces of wilted cotton plants, which were collected in the end of 1924-25 season. Twelve pots were filled with this soil ; 21 plants out of 48 developed the typical wilt disease. When the soil was resown with cotton in 1926, the percentage of wilt was raised to 61 from 44 of the previous year. In 1926, a fresh lot of " non-wilted " soil was mixed with pieces of wilted plants, collected in February and March of the same year; another lot of " non-wilted " soil was mixed with pieces of healthy dried plants, collected the same time as the wilted plants ; a third lot was at the same time mixed with pieces of dead branches of *Thespesia populnea*. These three different lots of the mixed soil were sown with cotton in June. Thirty-two per cent. of plants were typically wilted in the soil mixed with wilted plants ; 8 per cent. in the soil containing pieces of healthy dried cotton stems ; and in the third lot all the plants were healthy.

In 1927 these experiments were repeated, and once again similar results were obtained. Two modifications of these experiments were at the same time also tried. About fifty pieces from wilted plants of the previous season were externally sterilized and incubated a few days before sowing time. Every one of these pieces developed *Fusarium* Strain A. Having thus made sure that the fungus was viable in them, each piece was planted in direct contact with a cotton seed at the time of sowing. The germination of the seeds was very good and the seedlings developed into normal plants ; there were no cases of wilt. The other modification consisted in inoculating, with pure cultures of *Fusarium* Strain A, pieces of healthy dried cotton stems, collected in the end of the previous season. When the fungus was growing luxuriently on these stem pieces, they were mixed with the soil in the same way as wilted or healthy stems were mixed in the experiments described above. A few plants growing in these pots became wilted to the same extent as those growing in the soil mixed with pieces of healthy dried cotton stems.

Rosen's[1] experiments have shown that "in the presence of heavy infestations of the fungus," *Fusarium vasinfectum* Atk., nitrate of soda, at the rate of 1,000 pounds per acre, inhibits germination of cotton seed. In September 1928, *roseum* seeds were sown in pots containing either " wilted " or healthy soil or sand, to which

[1] Rosen, H. R. A consideration of the pathogenicity of the cotton wilt fungus, *Fusarium vasinfectum*. *Phytopath*, XXVIII, pp. 419—438, 1928.

was added sodium nitrate at the same rate used by Rosen. The soil or sand of some of these treated pots and of the untreated control pots was well mixed with *Fusarium* Strain A. The fungus was cultivated in 500 c.c. capacity flasks, each containing 300 c.c. of glucose agar medium. When the fungus was luxuriently growing in these flasks, the whole contents of one flask were added to each pot of one gallon capacity. The seeds germinated equally well in all the pots. Thus it was found that, unlike *F. vasinfectum*, *F.* Strain A does not inhibit germination in presence of sodium nitrate.

In September 1928, *roseum* was grown in pots of 1 gallon capacity containing either sand or " wilted " or healthy soil. To some of the pots of each of these three lots *Fusarium* Strain A was added before sowing the seed. The rest were kept as controls. There was another series identical in every way, except that the pots used were of three gallon capacity and therefore the amount of soil or sand in each pot was proportionately larger.

The fungus, that was to be used as an inoculum, was grown in Erlenmyre flasks of 500 c.c. capacity containing 300 c.c. of glucose agar; and when the fungus was growing luxuriently, the whole contents of a flask were added to each of the big or small pots to be inoculated and were well mixed with the soil or sand.

The seedlings growing in the small pots containing the inoculum began to die very rapidly. They did not show any signs of wilt, but there was a distinct collar rot. The seedlings at first were damping off only in those small pots which contained the fungus mixed with soil. Species of *Pythium* and *Phytophthora* were isolated from the diseased collar and leaves. There were also a few cases of *Rhizoctonia* in these pots. *Fusarium* Strain A was isolated from these diseased seedlings but chiefly along with the Phycomycetus fungi and *Rhizoctonia*. The few cases in which the *Fusarium* Strain A alone was isolated did not show any signs of wilt, but showed a clear wet rot and these seedlings were in an advanced stage of decay, when they were incubated. Presumably the *Fusarium* had overrun the Phycomycetus fungi and was therefore more readily isolated. A little later there were identical cases of damping off in the remaining small pots containing soil, serving as controls, and in the big pots, containing inoculated or uninoculated soil; but only a few plants had damped off and Phycomycetus fungi and *Fusarium* Strain A were isolated from these plants also, if they were not in an advanced stage of decay; but *Fusarium* Strain A alone was again isolated only from those plants which were wholly rotted and practically dead. Though only a few plants in these pots became diseased, still they became diseased in the same way as plants in the inoculated small pots. Plants growing in the pots containing sand remained healthy.

These experiments once again show that *Fusarium* Strain A is not parasitic and is present both in " wilted " and " non-wilted " soils. They further show the danger of adding a large quantity of a nutrient medium to the soil, especially when the weather is very wet, as was the case when these experiments were conducted.

In " wilted " soil, *rahar* (*Cajanus indicus*), gram, *Hibiscus esculentus*, tomatoes, and tobacco were sown. All these plants developed normally and remained healthy. Except *rahar* and gram, they were also sown in healthy soil, inoculated with the cotton wilt fungus ; none of them became infected.

The effect of water-logging on the incidence of wilt. Last season the effect of water-logging on wilt was tested by growing plants in pots which had no drain holes. In wilt affected soil, the incidence of wilt was 50 per cent., and in healthy soil it was 12 per cent., whereas in control pots, with proper drainage, the incidence of wilt was 29 per cent. in wilt affected soil and in healthy soil there was no wilt.

Plants grown in glass cases. In 1926 and 1927 plants in " wilted " and healthy soils were grown in glass cases ; some of these cases were placed under the tope of a tree and others were kept in the open so that they may be exposed direct to sunlight the whole day. The atmosphere inside all these cases was very humid, but the soil was never water-logged as water was given only when necessary : but the temperature inside the cases was much higher than that of the outside atmosphere. None of the plants growing in these cases developed wilt. They flowered and set fruit normally.

The effect of the size of pots on the incidence of wilt. In the course of the study of this disease during the last four years or so, it was often noticed that the incidence of wilt in pots of varying capacities was not the same, even though the pots were filled with the same lot of " wilted " soil. There appeared to be some evidence to suspect that there was less wilt in small pots than in big pots. Therefore experiments were started to see how far this suspicion was justified. In 1927, pots of six different capacities were filled with the same lot of " wilted " soil ; there were six pots of each size ; they were planted with AK2 varieties. In 1928 the experiment was repeated ; but, as AK2 seeds were not available, this variety was replaced by *Roseum*. The results are given in Tables I and II.

TABLE I.

1927—variety AK2

Capacity of pots in gallons	Percentage of wilt
18	29
8	20
$4\frac{1}{2}$	12
$2\frac{1}{2}$	41
$1\frac{1}{4}$	8
$\frac{1}{8}$	4

TABLE II.

1928—variety Roseum.

Capacity of pots in gallons	Percentage of wilt
18	85
8	85
$4\frac{1}{2}$	76
$2\frac{1}{2}$	56
2	64
$\frac{5}{8}$	12

If we refer to Table I, we find that for some unknown reason pots of $2\frac{1}{2}$-gallon capacity have given the largest amount of wilt. If we exclude this result, it seems from these two experiments that the size of pots has some influence on the incidence of wilt.

WATER CULTURE EXPERIMENTS.

It was found from preliminary trials that cotton seedlings grew very well in Knop's solution of half the normal strength ; and so, for water culture experiments, this was the medium used. Seedlings were first grown in sand for about a week and then transferred to tall Jena glass jars containing Knop's solution of half the normal strength. The solution was changed once every week. The seedlings were allowed to grow in this solution for two to three weeks, by which time the root system was well developed and the seedlings had put forth new leaves. In the third or the fourth week, some of the jars were filled with equal quantities of Knop's so ution and aluminium nitrate N/900 so ution or aluminium nitrate N/600 solution. These solutions were changed once every week. The plants growing in Knop's solution of half the normal strength flourished, but those growing in the solution containing aluminium nitrate had very poor growth. There was very little development of new roots and new leaves, especially when the higher strength of the aluminium salt was used. The plants in the solution with the aluminium salt died earlier than those in the pure Knop's solution in which flowers and bolls were developed. In the plants growing in the medium containing aluminium salt solutions, there was just a slight browning of the vascular tissues near the pith. In some jars, spores of *Fusarium* Strain A were regularly added at the time of the weekly renewal of the solutions, after the plants had been growing in these solutions for four or five weeks. But none of the plants took the infection.

In 1926 these experiments were repeated with this modification that a higher strength of aluminium nitrate solution was also used, *viz.*, N/300.

AK2 and AK4 plants growing in solutions containing equal parts of the standard Knop's solution and of aluminium nitrate solutions of different strengths showed internal browning which was more conspicuous in plants growing in solutions containing aluminium nitrate N/300. Plants growing in these media were stunted and did not develop many new roots or leaves; whereas those growing in half Knop's solution put forth a good growth and developed flowers and fruits (Plate III, figs. 1 and 2, Plate IV, figs. 1 and 2, Plate V, fig. 1). AK2 and AK4 plants growing in half Knop's solution or in any of the above mentioned mixtures containing aluminium salt solutions of different concentrations did not get the infection when spores of *Fusarium* Strain A were regularly added at the time when the old solutions were changed for fresh ones.

Plants growing in these Knop's solutions containing aluminium salts showed more or less typical wilt symptoms and these symptoms were more marked, higher the strength of the aluminium salts. Microscopic characters of these plants were the same as those of naturally wilted plants and they gave the same microchemical reactions when treated with logwood and ammonium carbonate or Alizarin or Brazilin.

Plants of Buri did not wilt in Knop's solution to which was added aluminium nitrate in varying proportions. The plants appeared to die of starvation, but there was no browning of the tissues and no pink reaction with Alizarin.

Staling Experiments.

In 1925, 1926 and 1927, several experiments were conducted to see on cotton plants the effect of substances secreted or excreted by some fungi. The staling products of the following *Fusaria* were tried:—

Fusarium isolated from "wilted" and "non-wilted" soils and wilted cotton plants; identical to *Fusarium* Strain A, described by Jiwan Singh[1].

Fusarium isolated from "wilted" and "non-wilted" soils and wilted cotton plants; identical to *Fusarium* Strain B, described by Jiwan Singh.

Fusarium isolated from wilted cotton plants left standing in the fields a long time after they are dead. Its conidia form blue incrustations on the underground decaying parts of the plant. The fungus is identical to *Fusarium* Strain D, described by Jiwan Singh.

Fusarium sp. isolated from wilted cotton plants in the South Karnatak.[2]

Fusarium sp. isolated from a decaying cotton boll.

Fusarium sp. isolated from dead wheat plants.

Fusarium sp. isolated from dead rice plants.

[1] Jiwan Singh. Loc. cit.

[2] This fungus was kindly supplied by Mr. G. S. Kulkarni, Special Cotton Mycologist, Dharwar. It has been found by Jiwan Singh to be identical to his *Fusarium* Strain A.

Fusarium vasinfectum Atk.[1]
Fusarium udum Butl. isolated from wilted *Rahar* plants.
Fusarium sp. isolated from wilted gram plants.

In addition to the staling products of these *Fusaria*, those of *Rhizoctonia* sp. from cotton, and of *Mucor* sp., *Penicillium* sp., and *Aspergillus* sp. isolated from "wilted" cotton soils were also tried.

Each of these fungi was subcultured in Erlenmyer flasks of 200 c.c. capacity, each containing 50 c.c. either of liquid potato extract [2] or of Richard's solution. When the cultures were a month or more old, the liquid from each lot of these flasks was decanted and filtered either under pressure through six to eight folds of filter paper or through Allundum filtering cones. The filtrate was divided, in each case, in two parts—one part was used as it was, and the other was just brought to the boil and then allowed to cool to room temperature before being used. Seedlings and cuttings of AK2 and AK4 plants were used for these experiments. The cuttings were cut under water and were more or less of the same size. The seedlings were raised in sand; as far as possible seedlings with the same number of leaves were selected. Each seedling or cutting was placed in a glass tube containing 15 c.c. of the filtrate. Control seedlings and cuttings were similarly kept in tubes, either containing 15 c.c. of distilled water or of sterilized Richard's solution or of sterilized liquid potato extract.

The filtrates of all these fungi gave practically the same results. They were, more or less, all toxic to cotton seedlings and cuttings. The results in detail are given in Tables III to X.

TABLE III.

Staling experiment No. I. (26th October, 1925).

Cuttings and seedlings of AK2 and AK4 placed in the staled products from one month's old cultures on liquid potato extract.

Fungus *Fusarium*	Filtrate	Plants	No. of hours when wilt first appeared		No. of hours when the plant completely wilted	
			Cuttings	Seedlings	Cuttings	Seedlings
1. Strain A (from a wilted plant).	Unboiled .	AK2	6	12	19	20
	Boiled .	AK2	3	6	8	19
	Unboiled .	AK4	5	5	8	19
	Boiled .	AK4	4	2·5	7·5	12

[1] This fungus was obtained, through the kindnes of Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, London, from Dr. Shear of the Bureau of Plant Industry, U. S. A.

[2] This medium was made up as follows:—
Potatoes were peeled and cut into small pieces and thoroughly washed. 300 grams of these pieces were stewed in a litre of water for two hours. Fifty c.c. of the liquid extract, which was obtained by straining the stew through fine muslin, were sterilized, in Erlenmyer flasks, of 200 c.c. capacity each, at 120°C. for 20 minutes.

TABLE III—*concld.*

Staling experiment No. I. (26th October, 1925)—*concld.*

Fungus *Fusarium*	Filtrate	Plants	No. of hours when wilt first appeared		No. of hours when the plant completely wilted	
			Cuttings	Seedings	Cuttings	Seedings
2. Strain A (from "wilted" soil).	Unboiled	AK2	3	2	8	4·5
	Boiled	AK2	4·5	7·5	19	19
	Unboiled	AK4	8	6	19	19
	Boiled	AK4	8	6	19	19
3. Strain A (from "non-wilted" soil).	Unboiled	AK2	19	2·5	24	12
	Boiled	AK2	4	6	10	19
	Unboiled	AK4	2·5	1·5	19	7·5
	Boiled	AK4	2·5	4	7·5	10
4. Strain B (from a wilted plant).	Unboiled	AK2	5	1·5	10	8
	Boiled	AK2	10	7·5	19	19
	Unboiled	AK4	12	12	20	20
	Boiled	AK4	12	5	20	19
5. Strain B (from "wilted" soil.)	Unboiled	AK2	12	7·5	19	19
	Boiled	AK2	5	4	12	12
	Unboiled	AK4	2·5	2·5	8	7·5
	Boiled	AK4	1·5	1·5	7·5	12
6. Strain B (from "non-wilted" soil).	Unboiled	AK4	5	4	10	19
	Boiled	AK2	6	4·5	19	12
	Unboiled	AK4	6	4·5	19	7·5
	Boiled	AK4	2·5	4	5	19

Controls in distilled water were healthy when the experiment ended on the fourth day.

TABLE IV.

Staling experiment No. 2. (9th December, 1925).

Cuttings of AK2 and AK4 plants placed in the staled products from two months' old cultures on Richard's solution.

Fungus *Fusarium*	Filtrate	Plants	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. Strain A (from a wilted plant)	Unboiled	AK2	24	30
	Boiled	AK2	4·5	9
	Unboiled	AK4	4·5	24
	Boiled	AK4	23·5	28
2. Strain A (from "wilted" soil)	Unboiled	AK2	7·5	10
	Boiled	AK2	7·5	10
	Unboiled	AK4	7·5	9
	Boiled	AK4	7·5	9
3. Strain A (from "non-wilted" soil)	Unboiled	AK2	7·5	9
	Boiled	AK2	7·5	9
	Unboiled	AK4	23	30
	Boiled	AK4	24	30
4. Strain B (from a wilted plant)	Unboiled	AK2	7·5	24
	Boiled	AK2	7·5	24
	Unboiled	AK4	19·5	24
	Boiled	AK4	9	21
5. Strain B (from "wilted" soil)	Unboiled	AK2	2·5	7·5
	Boiled	AK2	7·5	12
	Unboiled	AK4	24	30
	Boiled	AK4	2·5	7·5
6. Strain B (from "non-wilted" soil)	Unboiled	AK2	7·5	12
	Boiled	AK2	19·5	24
	Unboiled	AK4	4·5	9
	Boiled	AK4	19·5	24

Controls in distilled water were healthy when the experiment ended on the second day.

TABLE V.

Staling experiment No. 3. (6th January, 1926).

Cuttings of AK2 and AK4 plants placed in the staled products from 1½ months' old cultures on liquid potato extract.

Fungus *Fusarium*	Filtrate	Colour of the filtrate	Plants	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. Strain A from a wilted plant	Unboiled	Dark straw yellow transparent	AK2	4·5	8
	Boiled		AK2	4·5	8
	Unboiled		AK4	20	26
	Boiled		AK4	4	7
2. Strain A from "wilted" soil	Unboiled	Straw yellow trans-parent.	AK2	4	6·5
	Boiled		AK2	24	30
	Unboiled		AK4	24	30
	Boiled		AK4	4	6·5
3. Strain B from a wilted plant	Unboiled	Light straw-yellow slightly opaque.	AK2	12	20·5
	Boiled		AK2	12	20·5
	Unboiled		AK4	12	20·5
	Boiled		AK4	12	20·5
4. Strain B from "non-wilted," soil	Unboiled	Light straw slightly opaque.	AK2	2	5
	Boiled		AK2	12	20·5
	Unboiled		AK4	3·5	6·5
	Boiled		AK4	12	20·5
5. Strain B from wilted plant	Unboiled	Dark straw yellow trans-parent.	AK2	3	6
	Boiled		AK2	3	6·5
	Unboiled		AK4	20·5	30
	Boiled		AK4	5	7
6. From Rice	Unboiled	Dark straw yellow slightly turbid.	AK2	6·5	20·5
	Boiled		AK2	3	6·5
	Unboiled		AK4	20·5	24
	Boiled		AK4	3·5	6·5
7. From wheat	Unboiled	Light straw yellow slightly opaque.	AK2	3	6·5
	Boiled		AK2	12	20·5
	Unboiled		AK4	6·5	12
	Boiled		AK4	3·5	6

Controls in distilled water and liquid potato extract were healthy when the experiment ended on the second day.

TABLE VI.

Staling experiment No. 4. (21st January, 1926).

Cuttings of AK2 and AK4 plants placed in the staled products from 1¼ months old cultures on Richard's solution.

Fungus *Fusarium*	Filtrate	Plant	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. Strain A from a wilted plant	Unboiled	AK2	4·5	21
	Boiled	AK2	4·5	9
	Unboiled	AK4	*	*
	Boiled	AK4	12	21
2. Strain A from "wilted" soil	Unboiled	AK2	12	21
	Boiled	AK2	3·5	21
	Unboiled	AK4	12	21
	Boiled	AK4	3·5	21
3. Strain A from "non-wilted" soil	Unboiled	AK2	*	*
	Boiled	AK2	12	21
	Unboiled	AK4	1	5
	Boiled	AK4	21	30
4. Strain B from a wilted plant	Unboiled	AK2	12	21
	Boiled	AK2	12	21
	Unboiled	AK4	12	21
	Boiled	AK4	12	21
5. Strain B from "wilted" soil	Unboiled	AK2	4·5	21
	Boiled	AK2	4·5	21
	Unboiled	AK4	4·5	21
	Boiled	AK4	*	*
6. Strain B from "non-wilted" soil	Unboiled	AK2	2	5
	Boiled	AK2	4·5	21
	Unboiled	AK4	12	21
	Boiled	AK4	4·5	21
7. Strain D from a wilted plant	Unboiled	AK2	12	21
	Boiled	AK2	4·5	21
	Unboiled	AK4	12	21
	Boiled	AK4	4·5	21
8. From Rice	Unboiled	AK2	12	21
	Boiled	AK2	4·5	21
	Unboiled	AK4	12	21
	Boiled	AK4	4·5	21
9. From wheat	Unboiled	AK2	12	21
	Boiled	AK2	12	21
	Unboiled	AK4	2	21
	Boiled	AK4	3·5	21

Controls in distilled water and Richard's solution were healthy when the experiment ended.

*These plants showed no signs of wilt till the time when the experiment ended on the 2nd day.

TABLE VII.

Staling experiment No. 5. (13th July, 1926).

Seedlings of AK2 and AK4 placed in the staled products from $2\frac{1}{2}$ months' old cultures on Richard's solution.

Fungus *Fusarium*	Filtrate	Colour of filtrate	Plants	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. From Dharwar (identical to Strain A).	Unboiled	Light straw	AK2	20	24
	Boiled		AK2	20	24·5
	Unboiled	yellow clear.	AK4	16	22·5
	Boiled		AK4	20	22·5
2. Strain B from a wilted plant	Unboiled	Light straw yellow very slightly turbid.	AK2	3·5	22·5
	Boiled		AK2	3·5	20
	Unboiled		AK4	5	20
	Boiled		AK4	22·5	25
3. Strain C from a wilted plant	Unboiled	Light straw yellow slightly turbid.	AK2	12	20
	Boiled		AK2	19	22·5
	Unboiled		AK4	12	20
	Boiled		AK4	20	25·5
4. Strain D from a wilted plant	Unboiled	Light amber clear.	AK2	3·5	19
	Boiled		AK2	18	24
	Unboiled		AK4	5	22·5
	Boiled		AK4	19	22·5
5. *F. vasinfectum* Atk. from U. S. A.	Unboiled	Light straw yellow slightly turbid.	AK2	19	22·5
	Boiled		AK2	19	22·5
	Unboiled		AK4	16	24
	Boiled		AK4	19	24
6. Strain H from a dead cotton boll.	Unboiled	Light straw yellow slightly turbid.	AK2	3·5	22
	Boiled		AK2	*	*
	Unboiled		AK4	16	22
	Boiled		AK4	*	*
7. *F. udum* Butl. from a wilted *Arhar* plant.	Unboiled	Straw yellow clear.	AK2	16	22
	Boiled		AK2	16	22
	Unboiled		AK4	15	19·5
	Boiled		AK4	16	22
8. From wilted grain . .	Unboiled	Light amber clear.	AK2	3·5	19
	Boiled		AK2	3·5	24
	Unboiled		AK4	19	22·5
	Boiled		AK4	19	22·5

Controls in distilled water and Richard's solution were healthy when the experiment ended.

* These plants showed no signs of wilt till the time when the experiment ended on the 2nd day.

TABLE VIII.

Staling experiments Nos. 6A. and 6B.

Cuttings of AK2 and AK4 plants placed in the staled products from 1½ months' old culture on Richard's solution.

Experiment No. 6A was conducted on the 4th September 1926 and Experiment No. 6B was conducted on the 15th November 1926.

Fungus *Fusarium*	Filtrate	Colour of filtrate	Plants	No. of hours when wilt first appeared		No. of hours when the plant completely wilted	
				Exp. 6A	Exp. 6B	Exp. 6A	Exp. 6B
1. From Dharwar (identical to Strain A.)	Unboiled	Light straw	AK2	1	28·5	5	48
	Boiled		AK2	19	25·5	24	48
	Unboiled	yellow	AK4	19	28	24	47·5
	Boiled	clear.	AK4	19	4·5	24	28·5
2. Strain B from a wilted plant.	Unboiled	Light pale straw	AK2	*	20·5	[*]	48
	Boiled		AK2	19	2·5	24	28·5
	Unboiled	yellow	AK4	1	6	8	31
	Boiled	clear.	AK4	*	3	[*]	29·5
3. Strain C from a wilted plant.	Unboiled	Light pale straw	AK2	19	20·5	24	28·5
	Boiled		AK2	19	12	24	24·5
	Unboiled	yellow	AK4	1	12	24	24·5
	Boiled	clear.	AK4	19	20·5	24	25·5
4. Strain D from a wilted plant.	Unboiled	Dark amber clear.	AK2	12	20	20	28·5
	Boiled		AK2	19	*	24	*
	Unboiled		AK4	19	*	24	*
	Boiled		AK4	19	*	24	*
5. *F. vasinfectum* Atk. from U. S. A.	Unboiled	Very light straw yellow	AK2	*	20	*	28·5
	Boiled		AK2	1	20·5	8	28·5
	Unboiled		AK4	1	20·5	8	28
	Boiled		AK4	2	24·5	24	28·5
6. Strain H from a dead cotton boll.	Unboiled	Light pale straw yellow	AK2	1	23·5	8	28·5
	Boiled		AK2	3	20·5	24	[48]
	Unboiled	slightly turbid.	AK4	19	20·5	24	47·5
	Boiled		AK4	4	20·5	24	28·5
7. *F. udum.* Butl. from wilted *Arhar* plant.	Unboiled	Light straw yellow clear.	AK2	19	40	24	50
	Boiled		AK2	19	2	24	28·5
	Unboiled		AK4	19	32	24	42·5
	Boiled		AK4	19	24·5	24	28·5
8. From wilted gram	Unboiled	Darkamber clear.	AK2	19	24·5	24	28·5
	Boiled		AK2	4	20·5	12	52·5
	Unboiled		AK4	3	28·5	12	48
	Boiled		AK4	19	2	24	24

Controls in distilled water and Richard's Solution were healthy when the experiment ended.

* These plants showed no signs of wilt till the time when the experiment ended on the 2nd day in the case of Experiment No. 6A and on the 3rd day in the case of Experiment No. 6B.

TABLE IX.

Staling experiment No. 7. (7th October, 1927).

Cuttings of AK2 placed in the staled products from one month's old cultures on Richard's solution. This experiment was repeated twice.

Fungus	Filtrate	Colour of the filtrate	Cutting No.	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. From Dharwar (identical to *Fusarium* Strain A.)	Unboiled	Straw yellow clear.	1	20·5	36
	,,		2	16	30
	Boiled		1	20·5	34
	,,		2	12	29·5
2. *Fusarium* Strain A from a wilted plant.	Unboiled	Straw yellow clear.	1	5·5	30
	,,		2	16	36
	Boiled		1	12	29·5
	,,		2	20·5	36
3. *Fusarium* Strain B from a wilted plant.	Unboiled	Light pale straw yellow clear.	1	10	20
	,,		2	10	20
	Boiled		1	10	20
	,,		2	10	20
4. *Fusarium* Strain D from a wilted plant.	Unboiled	Dark amber.	1	12	24·5
	,,		2	12	29·5
	Boiled		1	20·5	36
	,,		2	20·5	36
5. *Fusarium vasinfectum* Atk. from U. S. A.	Unboiled	Straw yellow clear.	1	24·5	36
	,,		2	24·5	36
	Boiled		1	20·5	36
	,,		2	20·5	29·5
6. *Fusarium* from wilted gram	Unboiled	Light straw yellow clear.	1	20·5	36
	,,		2	5·5	20·5
	Boiled		1	12	24·5
	,,		2	12	24·5
7. *Fusarium udum* Butl. from wilted *Arhar* plant.	Unboiled	Straw yellow clear.	1	20·5	36
	,,		2	20·5	36
	Boiled		1	12	29·5
	,,		2	24	36
8. *Rhizoctonia* on cotton	Unboiled	Colourless	1	1·5	29·5
	,,		2	1·5	24·5
	Boiled		1	5	20·5
	,,		2	5	29·5

Controls in distilled water and Richard's solution were healthy when the experiment ended on the third day.

TABLE X.

Staling experiment No. 8. (10th November, 1927).

Cuttings of AK2 placed in the staled products, from 1½ months' old cultures on Richard's solution. This experiment was repeated six times.

Fungus	Filtrate	Colour of the filtrate	Cutting No.	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
1. *Fusarium* Strain A from wilted soil.	Unboiled	Light straw yellow.	1	5·5	22
	,,		2	7	24
	,,		3	12	24
	,,		4	12	22
	,,		5	2	8·5
	,,		6	12	22
	Boiled		1	2	8·5
	,,		2	3·5	22
	,,		3	8·5	22
	,,		4	5·5	22
	,,		5	2	8·5
	,,		6	2	8·5
2. *Mucor* sp. from wilted soil	Unboiled	Very light straw yellow.	1	6	24
	,,		2	2	24
	,,		3	22	†
	,,		4	22	†
	,,		5	22	†
	,,		6	12	†
	Boiled		1	12	24
	,,		2	12	24
	,,		3	12	24
	,,		4	8·5	22
	,,		5	8·5	24
	,,		6	2	8·5

† These plants were not completely wilted when the experiment ended on the 2nd day.

TABLE X—*contd.*

Stating experiment No. 8. (10th November, 1927)—*contd.*

Fungus	Filtrate	Colour of the filtrate	Cutting No.	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
3. *Penicillium* sp. from wilted soil.	Unboiled	Black	1	4	8·5
	,,		2	2	8·5
	,,		3	5·5	22
	,,		4	12	24
	,,		5	5·5	22
	,,		6	7·5	22
	Boiled		1	22	†
	,,		2	8·5	22
	,,		3	2	22
	,,		4	12	22
	,,		5	3·5	22
	,,		6	6	8·5
4. *Penicillium* sp. from wilted soil.	Unboiled	Dark straw yellow.	1	2	8·5
	,,		2	2	8·5
	,,		3	5·5	22
	,,		4	12	†
	,,		5	12	†
	,,		6	12	†
	Boiled		1	6·5	24
	,,		2	5·5	22
	,,		3	*	*
	,,		4	*	*
	,,		5	*	*
	,,		6	5·5	22

* These plants showed no signs of wilt till the time when the experiment ended on the 2nd day.
† These plants were not completely wilted when the experiment ended on the 2nd day.

TABLE X—*concld.*

Staling experiment No. 8. (10th November, 1927)—*concld.*

Fungus	Filtrate	Colour of the filtrate	Cutting No.	No. of hours when wilt first appeared	No. of hours when the plant completely wilted
5. *Aspergillus* sp. from wilted soil	Unboiled	Orange straw yellow.	1	2	8·5
	,,		2	2	8·5
	,,		3	5·5	22
	,,		4	5·5	22
	,,		5	12	22
	,,		6	8·5	22
	Boiled		1	2	8·5
	,,		2	2	22
	,,		3	5·5	22
	,,		4	3·5	8·5
	,,		5	3·5	8·5
	,,		6	3·5	8·5

Controls in distilled water and Richard's solution were healthy when the experiment ended.

DISCUSSION AND CONCLUSIONS.

It may be urged that because a particular *Fusarium* has been found in wilted plants, therefore it must be pathogenic and directly responsible for the disease. The more or less constant association of a fungus with a diseased plant does not necessarily connote its parasitism. For example, *Phoma beta* is constantly associated with the leaf rot of sugar beet and beet root. Gäumann[1] considers that there are two distinct causes of the beet rot, the primary cause being physiological and the fungus being only secondary. A *Fusarium* is also constantly associated with the brown rootrot of tobacco, but its parasitism has not been definitely demonstrated and Johnson, Slagg and Murwin[2] are of opinion that evidence points strongly in the direction of the disease being not due to the *Fusarium*.

[1] Gäumann, E. Untersuchungen über die Herzkrankheit (Phyllonekrose) der Runkel-und Zuckerrüben. Beibl. zur Vierteljahrsschr. Naturforsch. Geschlsch. Zürich, LXX, No. 7, 1925.

[2] Johnson, J., Slagg, C. M., and Murwin, H. F. The brown root-rot of tobacco and other plants. *U. S. Dept. Agri. Bull.* 1410,1926.

In connection with Western Yellow Blight of tomatoes, there is the almost invariable occurrence of root decay, and several fungi, principally *Fusarium* spp. and *Rhizoctonia solani*, have been isolated from the rotting roots, but no conclusive or consistent results have been obtained to prove the parasitism of these fungi. Shapovalov[1] has come to the conclusion, as a result of his experiments, that this blight is "most decidedly determined by certain combinations of weather conditions" and that the fungus attack may be a secondary phenomenon.

As pointed out by Johnson and his collaborators *Fusarium* spp., being soil saprophytes, may be found in decaying and healthy roots. In tobacco plants *Fusarium* sp. has been isolated from healthy roots of not only susceptible varieties but also of immune varieties. *Fusarium* spp. have also been obtained both from healthy and diseased cotton plants. We have already seen that healthy mature cotton plants, both of susceptible and immune varieties, growing in wilted soil have the fungus in their healthy roots at the end of the season. In healthy plants growing in non-wilted soil treated with *nim* cake, the fungus has also been found in normal parts of the tap root above the gall. Plants even of immune varieties injected with solutions of aluminium salts develop typical wilt, and at first the wilted plants do not show any trace of the fungus, but if the dying injected plants are left in the soil for some time, the fungus is found in them.

Typical wilt has also been produced by heavily manuring non-wilted soil. When aluminium salts are added to non-wilted soil, some plants become wilted. When *Fusarium* Strain A is added to the aluminium treated soil, the percentage of wilt is increased. Wilt is also produced in plants growing in Knop's solution to which aluminium salts have been added, but if fungus spores are added to the nutrient solution, the plants do not become infected with the fungus, even if they are wilting. Plants grown in pots in which the soil is kept water logged become typically wilted. But plants grown in healthy soil to which has been added *Fusarium* Strain A have never produced the typical wilt symptoms; this fungus has been commonly isolated from wilted plants in these Provinces and is considered by Jiwan Singh[2] to be identical with the *Fusarium*, which, according to Kulkarni[3], is the cause of cotton wilt in the South Karnatak. Thus we see that, under certain conditions, all the pathological symptoms of typical wilt can be produced without the addition of the fungus, commonly associated with naturally wilted plants, or of its toxins.

Old mature plants in wilted soil show internal signs of wilt; for example there is typical browning of the walls of the xylem cells and plugging of vessels and cells with some brown granular substances (Plate VI, fig. 3); there are also formation of tyloses in the vessels and excessive starch development, especially in the medullary

[1] Shapovalov, M. Ecological aspects of a pathological problem (Western Yellow Blight of Tomatoes). *Ecology*, VI, No. 3, pp. 241—259, 1926.
[2] Jiwan Singh. Loc. cit.
[3] Kulkarni, G. S., Loc. cit.

rays (Plate VI, fig. 7); but the browning of the cell walls and plugging of the lumen are very limited and not as much as in wilted plants and that is the reason why the mature plants do not show any signs of wilt. All these internal wilt symptoms in mature plants may be found with or without the presence of the fungus in the tissues. The amount of fungus, if present, is not much less than in wilting plants. Wilt therefore seems to be premature senility. The young plants are aggregating or precipitating in their tissues the same substances as the mature plants, but to a greater degree.

In Egypt, Fahmy[1] also has found that externally healthy plants show typical internal wilt characteristics, but he does not mention at what stage in the development of these healthy plants there is the internal discolouration, nor does he say to what extent the tissues are discoloured.

In dying plants and seedlings, *Fusarium* Strain A has never been found, unless associated either with the internal and external wilt symptoms or with *Rhizoctonia* or *Pythium* or *Phytophthora* which have been known to kill plants and seedlings without the aid of this *Fusarium ;* but it has been found in healthy plants without being pathogenic to them.

Wilt has not been found to be the result of mechanical plugging of the xylem vessels by the fungus that may be found in wilting plants. The amount of fungus present in a wilt affected plant, as seen in transverse sections bears no relationship to the wilting stage of the plant. Sections from a plant showing early signs of wilt may show more or less the same amount of fungus as in those of a badly wilted plant. The number of cells in which the fungus is found is very small compared to the number of cells in which there is no trace of the fungus and even when the mycelium is present, the cell lumen is not always completely choked. Ajrekar and Bal,[2] Rosen[3] and others have also noted the scarcity of fungus mycelium in the tissues of a wilted plant.

Rosen made experiments to find means by which the American cotton wilt fungus, *Fusarium vasinfectum* induces wilt. He grew the pathogen in nutrient media, especially Richard's solution, and in the filtrate were placed seedlings with their roots cut off. The results obtained by him from his staling experiments are more or less identical with those obtained by the writer. Besides *Fusarium vasinfectum*, Rosen had used *Fusarium Lycopersicum* and *Fusarium Trachiphilum ;* the filtrates from all these three *Fusaria* were found to be toxic to cotton plants. The writer has used for his experiments several species of *Fusaria, Rhizoctonia* sp., *Mucor* sp., *Aspergillus* sp. and *Penicillium* sp., isolated from cotton soils or wilt affected cotton plants; all of them have been found to produce toxins in their

[1] Fahmy, Loc. cit. *Phyto pat.*, XVII, pp. 749—767, 1927.
[2] Ajrekar and Bal. Loc. cit.
[3] Rosen, H. R. Efforts to determine the means by which the cotton wilt fungus, *Fusarium vasinfectum*, induces wilting. *Jour. Agr. Res.*, XXXIII, pp. 1143—1162, 1926

filtrates, which are capable of being poisonous to seedlings and cuttings of cotton plants placed in the filtrates.

From his studies, to Rosen " it seems logical to conclude that the pathological phenomena are due primarily to certain poisonous chemical compounds produced by the fungus". He came to this " logical conclusion ", in spite of the fact that he has " not infrequently been unable to culture the fungus from wilted plants which possessed discoloured vascular elements, even when stem bases and pieces of tap roots were utilized", and therefore suggests " that the wilting and internal discolouration of the xylem in these cases are due to the formation of toxic substances by the fungus in the soil".

To the writer it seems doubtful, if from the staling experiments, this general conclusion can safely be drawn. There would have been perhaps some justification for this conclusion, if Rosen had proved that *Fusarium vasinfectum* was confined only to wilt infected cotton soils and that cotton grown in soils not containing this fungus did not become wilted, and that in these soils wilt was produced by the introduction of the fungus. In absence of this evidence Rosen's conclusion cannot be accepted. In these Provinces we have seen that (1) there are four species of *Fusaria* isolated from wilted cotton plants, (2) three of these *Fusaria* have been isolated from cotton soils, both healthy and " wilted", (3) by the addition of these *Fusaria*, from wilted plants and wilt affected soils, to non-wilted soil, wilt is not induced in cotton plants, and (4) all these *Fusaria* are capable of producing substances which are toxic to cotton plants, when either seedlings or cuttings are placed in these toxins. Therefore any toxins or poisonous chemical substances that these *Fusaria* may be capable of producing will be produced both in healthy and " wilted " soils and therefore these toxins cannot be said to be the cause of cotton wilt. Again, from the results obtained by putting healthy cuttings and rooted plants in certain pure toxins to conclude that the wilting of plants growing under natural conditions is due to these toxins produced in the soil or in the plant tissues is to assume that under field conditions plants have no other substances to absorb accept the toxins in a pure form as in laboratory experiments. Another argument against the toxin theory is that when the filtrate from the *Fusarium* Strain A, grown in Richard's solution, is injected into healthy plants, it does not produce any toxic effect, though it is found to be toxic to cotton cuttings placed in it.

This " logical conclusion " of his does not seem to be now accepted even by Rosen himself[1], because, in his recent publication, he not only makes no mention of this toxic effect of the fungus on plants, but admits that though he conducted large numbers of experiments, over a period of about seven years, involving field and green house tests, he failed, with very few exceptions, to obtain the typical field forms of wilt ; he now believes " that nematodes, wire-worms or other insects, *Rhizoctonia* lesions, water levels which tend to asphyxiate roots, and other factors

[1] Rosen, H. R. A consideration of the pathogenicity of the cotton wilt fungus. *Phytopath*, XVIII, pp. 419—438, 1929.

which tend to destroy or break down root tissues or which inhibit normal root development render the plant susceptible to wilt".

Fahmy[1] has also found that the *Fusarium*, which he considers to be the cause of the cotton disease in Egypt, produces on modified Richard's solution a staling substance which was capable of causing cotton seedlings to wilt when they were placed in the filtrate of the culture; and therefore he considers it "possible that the parasite within the host is capable of producing similar staling substances which may under certain conditions cause the infected plants to wilt"; but it may also be possible that these "certain conditions" may be the real cause of wilt and not necessarily the staling substances.

Kulkarni and Mundkur,[2] from their "study of the effect on cotton plants of solutions in which *Fusarium vasinfectum* Atk., so closely associated with the wilt disease of cotton" in the Bombay Karnatak, have come to the conclusion that the "active factor causing wilt in cotton plants appears to be a chemical compound or compounds occurring in the liquid in which the fungus is grown". They found that the toxic substance is fatal even to immune varieties.

If the toxins, produced by the fungus found in wilt affected cotton plants, were really responsible for causing the disease, then they should be capable of producing the disease when injected into the tissues of healthy cotton plants; but from our injection experiments we have seen that the typical field form of wilt could be produced in plants injected with aluminium salt solutions, but when the plants were injected with spore suspensions of the fungus, or with its toxins produced in culture media, the plants remained healthy.

From the inoculation experiments made by the author[3] and Jiwan Singh[4], it is clear that *Fusaria* isolated from numerous wilted plants, which have been described by Jiwan Singh, under normal conditions have not been able to infect plants. The inoculations have been done in various ways, but they have given negative results except when *Fusarium* Strain A is introduced in wilted soil to which has been added aluminium salts; but plants become wilted in "non-wilted" soil as well when the soil is treated with these salts alone, though the percentage of wilt is less than in the former case. Jiwan Singh has recorded that in 1926 some of the cotton plants in his inoculation experiments were observed to be dying; the fungus inoculated was re-isolated from the underground parts of the dying plants, but the microscopic and microchemical characters of the dying plants were totally unlike wilt; and as the upper parts of the plants were attacked by *Rhizoctonia*, he is of opinion that these were not real cases of successful inoculations. The inoculated *Fusarium* had penetrated the plant tissues after the plant

[1] Fahmy, T. Loc. cit. *Phytopath.*, XVII, pp. 749—767, 1927.
[2] Kulkarni G. S., and Mundkur, B. B. III. The pathogeny of wilting in cotton plants. *Mem. Dept. Agri. India. Bot. Ser.*, XVII, pp. 21—27, 1928.
[3] Dastur, J. F. Loc. cit.
[4] Jiwan Singh. Loc. cit.

was weakened by an attack of *Rhizoctonia*. This conclusion of his is further strengthened by the fact that some of the control plants were also similarly affected and this particular *Fusarium* was also isolated from the subterranian parts of the uninoculated but dying plants and *Rhizoctonia* from the upper parts of the stem. Similar results were obtained by the writer as well, when the inoculum with a large amount of the nutrient medium, on which it was cultivated, was mixed with soil or sand in small pots of one gallon capacity, practically all the seedlings growing in the inoculated soil were diseased, whereas those growing in the inoculated sand were healthy. Most of the diseased seedlings had developed a wet rot and the remaining showed signs of seedling blight, caused by *Rhizoctonia*. If the seedlings in the early stage of disease were externally sterilized and incubated, Phycomycetus fungi, like *Pythium* and *Phytophthora*, were isolated from those that showed a wet rot and *Rhizoctonia* from the remaining. The inoculated *Fusarium* was also isolated along with these fungi from some of these seedlings. But the *Fusarium* alone was isolated only from those diseased plants which were in a very advanced stage of decay or rot; it is therefore not improbable that this *Fusarium* got into the tissues of the seedlings after they were attacked by the other fungi and overran them, when the seedlings were badly rotted. This suggestion is strengthened by the fact that in the control pots containing the uninoculated soils there were also a few cases of similarly diseased plants from which *Pythium*, *Phytophthora* and *Rhizoctonia* were isolated; *Fusarium* Strain A also was obtained from these diseased plants along with the other fungi, but the *Fusarium* alone was obtained when the seedlings were in a very advanced stage of decay.

These experiments clearly show the danger of jumping to the conclusion that the inoculations have been successful because some of the inoculated plants are dead or dying and the fungus inoculated has been re-isolated from them.

Butler[1] attempted to isolate the causal fungus from five wilted plants "in an early stage of the disease" which he collected from a field at Matargaon village in the Berars. From these five plants he got in cultures fungi only from three; the other two remained sterile. Of these three fungi, two were found to be identical, *Fusarium* sp. and the other was not identified, because it remained sterile. Thus we see that Butler also has found that in wilted plants, at least "in an early stage of the disease", a fungus is not invariably present and secondly, when present, it is not always the same. These findings of his would throw doubt on the fungus theory of wilt. In his inoculation experiments he got a few dying plants—plants which "died in the manner characteristic of *Fusarium* wilt". As already mentioned, cotton wilt has certain definite macroscopic and microscopic symptoms which are invariably found in the wilt affected plants; but these symptoms are not characteristic of all *Fusarium* wilts, such as the pigeon-pea wilt, *til* wilt, tobacco wilt, tomato

[1] Butler, E. J. The wilt diseases of cotton and sesamum in India. *Agri. Jour. India*, XXI, pp. 268—273, 1926.

wilt, etc., and therefore Butler's statement, that plants died in the manner "characteristic of *Fusarium* wilt," is vague and does not necessarily mean that the dying plants showed typical cotton wilt symptoms. He examined microscopically some of the inoculated dying plants and found in them fungus hyphæ and innumerable conidia. He does not record the presence of discoloured walls of xylem cells and the plugging of their lumen with a granular brown substance ; a careful observer, like Butler, would not have missed seeing these characteristics if they were present and therefore the supposition is permissible that there was no browning of the tissues, and that the xylem vessels and cells were normal except for the presence of "a dense matted" growth of hyphæ, but we have already seen the mere presence of the fungus does not prove its parasitism. Though he failed to get *Fusarium* from all wilt affected plants and he got only a low percentage of deaths from wilt in the inoculated plants, still he believes his experiments "show that the disease is caused by a *Fusarium*" which he admits is "capable of pathogenic action under certain conditions". The capricious results of his inoculations he attributes to the action of "some factor which aids or hinders infection by the fungi", but this factor according to him "cannot be a soil one", because of the fact that only one batch of soil was used in his experiments ; but if he used only one batch of soil he also used only one "batch" of the supposed pathogenic material. His conclusions therefore that the fungus is pathogenic and the soil factor non-existent are untenable.

Elliot[1] has found that even small quantities of the uninoculated nutrient medium, such as corn meal or cotton seed meal, make such a favourable medium for fungus and bacterial growth that plants in both the control pots and those inoculated with *F. vasinfectum* were killed by almost any organism which chanced to infect the meal. Butler for his experiments used suspensions in distilled water of the fungus. These suspensions would carry with them stray small quantities of the nutrient medium. Plants having their roots in close contact with the particles of the medium on which would be growing not only the inoculating material but also air born spores. These may affect the roots unfavourably, the plant may be killed and *Fusarium* hyphæ may penetrate the weakened tissues. This may account for Butler's results which he admits were capricious.

Kulkarni[2] has recently brought forward evidence to prove the parasitic nature of the *Fusarium* isolated from wilted cotton plants in the Bombay Karnatak ; however, he is of opinion that the "merely partial success of the parasitic fungus in killing plants suggests that some other condition is necessary to enable the parasite to attack and destroy cotton".

Every wilt affected plant, whatever be its age or the stage of the development of the disease, invariably has the walls of some parts of the xylem tissues discoloured yellow or brown or black, and, unless the disease has just commenced, some

[1] Elliot, J. A. Arkansas Cotton diseases. *Ark. Agri. Expr. Sta. Bul.* 7, No. 173, pp. 3—26, 1921.
[2] Kulkarni, G. S. Loc. cit.

of the xylem vessels and cells are blocked with some dark coloured granular substance. Fungus hyphæ, if present, are not necessarily found in these discoloured tissues and the tissues in which the fungus is present are not necessarily discoloured. These discoloured tissues are invariably stained blue with log-wood and ammonium carbonate, and beautiful red or pink with Alizarin or Brazilin. Hoffer and Carr[1] used the log-wood test for detecting microchemically aluminium in plant tissues. According to Tunmann,[2] the salts of this metal in plant tissues turn red with Alizarin or Brazilin. Since these reactions are exactly identical with those obtained when tissues of naturally wilted cotton plants are similarly treated; since these stains have no action on healthy tissues or tissues attacked by *Rhizoctonia* or *Phytophthora*; since typical internal and external wilt symptoms are reproduced when plants are grown in a healthy soil to which aluminium salts have been added or when healthy plants growing in a healthy soil are injected, either through petioles, twigs or roots, with solutions of aluminium salts; and since the tissues of these artificially wilt affected plants also give the typical reactions with the reagents mentioned above, it may be assumed that aluminium salts are present in the tissues of the wilt affected plants and cause blocking of the lumen of vessels and cells. When it is suggested that aluminium salts are accumulated in the vessels and cells of the xylem tissues, it does not necessarily imply that there is an increase in the total contents of the ions of the metal. The accumulation in cell lumen may be due to a chemical change taking place in the solution after it has entered the cell. However, the ash analysis* of wilted and healthy cotton plants, variety AK2 (Table XI), shows a considerable increase of alumina in wilted plants.

TABLE XI.

	% of Alumina	% of Iron oxide
Leaves of healthy plants	0·49	0·022
Leaves of wilted plants	0·964	0·0272
Stems of healthy plants	0·0864	0·0056
Stems of wilted plants	0·1786	0·0054

Because Hartwell and Pember[3], Hoffer and Carr[4] and others have shown that aluminium concentration causes a diseased condition of crops, such as

* These results were recently supplied by Mr. A. R. P. Aiyer, Agricultural Chemist to Government, Central Provinces, to whom I am indebted for analysing cotton plants for me.

[1] Hoffer, G. N., and Carr, R. H. Accumulation of aluminium and iron compounds in corn plants and its probable relation to rootrots. *Jour. Agri. Res.*, XXIII, pp. 801—823, 1923.

[2] Tunmann, O. Pflanzenmikrochemie, 1913.

[3] Hartwell, B. L., and Pember, F. R. The presence of aluminium as a reason for the difference in the effect of the so-called acid soil on barley and rye. *Soil. Sci.*, VI, pp. 259—279, 1918.

[4] Hoffer, G. N., and Carr, R. H. Loc. cit.

barley, rye and maize, in those soils which were positively acidic, having a pH-value of from 3·5 to 5·5; and because they have further found that these crops could be saved from the toxic effects of aluminium concentration in the soil by treating it with heavy doses of superphosphate and lime, Bal[1] assumes that the cotton soils of the Central Provinces and Berar, being alkaline, cannot exhibit aluminium toxicity. But Magistad[2] has adduced considerable evidence to prove that highly alkaline soils may also exhibit aluminium toxicity, because their soil solutions may contain an appreciable concentration of aluminate ion (AlO2 or AlO3). If Magistad's results are to be relied upon, then it is not surprising that (1) the addition of lime and superphosphate to the alkaline wilt affected soils of these Provinces has no inhibiting influence on cotton wilt, (2) healthy soils become susceptible to wilt when made more alkaline by the addition of lime and (3) " sick " soil develops less wilt when an acid is added to it. Because Bal failed to get even traces of soluble aluminium from " some water extracts from the normal and wilt producing soils ", therefore he assumes that cotton wilt cannot be due to aluminium toxicity. He evidently holds the view that elements are absorbed only in true solutions as simple ions, which view has been exploded by Comber [3], who has produced evidence to show that " matter in a state of high colloidal dispersion may actually be absorbed by plants". Recent researches have further indicated that " complex co-ordinated metal anions may readily penetrate and suffer translocation "; among the mobile complex metallic anions, Hardy[4] mentions alumino-oxalate ion, Al (C2O4)3, and ions of ferro compounds. Comber[5] and Reed and Haas[6] have shown that iron and aluminium may apparently form organo compounds and may sometimes be taken up by plant roots. Hardy makes the following statement, " perhaps the formation of this type (*viz.*, organo compounds) may account for the uptake of iron and aluminium from soils rich in organic matter, even in those cases when the presence of excessive calcium carbonate confers neutral or alkaline reaction on the soil". We have seen that healthy cotton soils though alkaline still when treated heavily with organic manures, such as oil cake and farm yard manure, produce wilt in cotton plants. These results are consonant with the statement made by Hardy. Further it appears, according to Hardy, that even colloidal complexes of aluminium or iron with certain acid anions, particularly organic acid anions, may penetrate plant cells, and therefore the organic acids that commonly occur in soil peptise alumina and

[1] Bal, D. V. Cotton wilt in Central Provinces and Berar, *Jour. Indian Bot. Soc.*, V, pp. 117—120, 1926.

[2] Magistad, O. C. The aluminium content of the soil solution and its relation to soil reaction and to plant growth. Soil. Sci. XX, pp. 181—226, 1925.

[3] Comber, N. M. The availability of mineral plant food. *Jour. Agri. Sc.*, XII, pp. 363—369, 1922.

[4] Hardy, F. The rôle of aluminium in soil infertility and toxicity, *Jour. Agri. Sc.*, XVI, pp. 616—631, 1926.

[5] Comber, N. M. Loc cit.

[6] Reed, H. S. and Haas, A. R. C. Iron supply in a nutrient medium. *Bot. Gaz.*, LXXVII, pp. 290—299, 1924.

hydrous ferric oxide, so that the metals are rendered susceptible of absorption by roots. Jones [1] has shown that the translocation of alumina and iron within the plant body appears to be confined mainly to organo metallic compounds and to complex metallic anions. Hoffer and Carr [2] have found that these metallic anions are deposited at special tissue regions, such as the nodal plates of maize. McGeorge[3] has found similar deposition on the nodal plates of sugarcane. In wilted cotton plants plugging of cell lumen is more marked at the nodes of stems and at the junction of the laterals with the tap root (Plate VI, figs. 9 & 10). Hardy[4] suggests that the excessive accumulation of complex and metallic ions in special tissue regions lead to tissue disintegration and may predispose the plants to root-rots ; it is not improbable that the accumulations, which have been invariably found in wilt affected cotton plants, predispose them to root-rots, and *Fusaria*—one species particularly more than others—penetrate their tissues.

Bal[5] points out that " even if an excess of aluminium salts occur in wilted plants, this may simply be due to disturbance of the physiological processes caused by the fungus". This argument of his would have some force if in wilted plants the fungus was invariably present or whenever the fungus was found in the plants there was this " disturbance of the physiological processes", but we know that the fungus is not always present in wilt affected plants and in healthy plants this fungus has been found without causing a " disturbance of the physiological processes". Again this " disturbance of the physiological processes " has been known to be checked if a plant in the very early stage of wilt attack is transplanted from the wilt affected soil to healthy soil, and this " disturbance " is checked even in the presence of the fungus which has been found to be viable in the tissues of the plant, weeks after it was transplanted.

The results obtained from the experiments in which " sick " soil was mixed with healthy soil in varying proportions may perhaps point towards the parasitic nature of cotton wilt, although the incidence of the disease was approximately proportionate to the quantity of the wilted soil. Johnson, Slagg and Murwin[6] have had similar results, when working with the root-rot of tobacco. On the other hand, the results of the several parallel series in which the two soils were not mixed together but were either in layers, one above the other, or in contact with each other through large drain holes of the small earthen pots embedded in bigger pots, seem inexplicable on a parasitic basis. These experiments show that when roots of plants growing in " wilted " soil penetrate healthy soil and from which they draw most of their nourishment, they are less liable to wilt ; whereas, when the plants put forth roots from healthy soil into " wilted " soil, they

[1] Jones, H. W. The distribution of inorganic iron in plant and animal tissues. *Bioch. Jour.*, XIV, pp. 654—659, 1920.

[2] Hoffer, G. M. and Carr, R. H. Loc. cit.

[3] McGeorge, W. T. The influence of aluminium, manganese and iron salts on the growth of sugar canes and their relations to the infertility of acid island soils. *Hawaiian Sugar Planters' Assoc. Bull.* 49, pp. 1—95, 1925.

[4] Hardy, F. Loc. cit. [5] Bal, D. V. Loc. cit.

[6] Johnson, J., Slagg, C. M., and Murwin, H. F. Loc. cit.

begin to absorb the greater part of their food from " wilted " soil, they become more susceptible to wilt. If wilt were caused by an organism, the disease would be transmitted to plants in healthy soil from " wilted " soil and perhaps also from wilt affected plants, as the roots of these plants would be interlaced together, but we have seen that plants growing in pots containing healthy soil, in which were embedded small pots containing wilted soil, did not catch the infection, though some of the plants in these small pots had wilted and, further, plants in pots containing healthy soil embedded in " wilted " soil became wilted after their roots had penetrated into the " wilted " soil. We also find that certain fields may be, wholly or in parts, wilt affected, but the disease does not necessarily spread to the surrounding healthy fields or to healthy areas round the wilt affected patches in the same field.

Both pot culture experiments and the rotation series at Akola show that *jowar* has some controlling effect on wilt. Cotton growing in wilted soil in which *jowar* had been grown or cotton grown mixed with *jowar* is not much susceptible to wilt.

Plants have developed typical wilt when growing in healthy soil which has been heavily manured, or to which aluminium salts have been added, or which is kept water logged, or when they are injected with aluminium salt solutions. At first the fungus, *Fusarium* Strain A, is not to be found in plants wilting as a result of the injections, but the fungus is found in the tissues if injected plants are left in the soil for some time. These experiments show that wilt can be induced in plants without the introduction of the fungus, and secondly that the fungus usually found in wilted plants is present in the healthy soil as well. It is true that, by the addition of pure cultures to soil treated with aluminium salts, a higher percentage of wilt is obtained. The fungus, in this soil being more active than in the un-inoculated soil, is more readily able to infect plants suffering for aluminium toxicity. Buri, a totally resistant variety, was not susceptible to aluminium toxicity from the soil though the presence of this salt in a soluble form was detected in the cell contents of the cortical cells, when sections from plants grown in soil containing a higher dose of the salts were treated with Alizarin. It absorbed the salts in its tissues, but its normal physiological functions were not disturbed by them. In the case of plants injected with 2 per cent., 1 per cent. and 0·5 per cent. aluminium nitrate, Buri, however, showed signs of wilt. These experiments show that Buri may absorb from the soil aluminium salts, but its physiological processes are not disturbed as in the case of susceptible varieties ; but when high concentrations of the nitrate salt are injected into the plant, it behaves similarly to susceptible varieties.

Perhaps the fungal origin of wilt may find support in the development of wilt in the " non-wilt " affected soil in which were mixed cuttings of dry wilted cotton plants of the previous season. But soil in which healthy cotton plant cuttings were mixed also produced wilt, though to a much smaller extent than in the former case. Again when single pieces of diseased stems, in which *Fusarium* Strain A was found to be viable, were put in the soil in contact with the seed at the time of sowing, there were no cases of wilt. Pieces of healthy cotton stems of the previous season were

used as a medium for growing *Fusarium* Strain A and when these pieces with a luxurious growth of the fungus on them were mixed with the soil, similarly as wilt affected cotton stems were mixed, there were only a few cases of wilt as in the case of the soil mixed with healthy stems. Therefore the incidence of wilt cannot be explained solely on the basis of the use of infected stems. Johnson, Slagg and Murwin[1] have also found that by mixing diseased tobacco stems in healthy soil, tobacco plants grown in this soil become infected.

Summary.

1. A description of cotton wilt in the Central Provinces and Berar is given. The microscopic, macroscopic and microchemical characters are very significant in identifying the disease, and distinguishing it from seedling blight, caused by *Rhizoctonia*, which is a very common and serious disease of seedlings.
2. It is shown that wilt affected plants have the same internal symptoms as the mature healthy plants growing in diseased soil.
3. *Fusarium* hyphæ are not necessarily always found in wilt affected plants, but have been found at times even in healthy plants.
4. There are some important points of differences between the cotton wilt under study and the American and Egyptian wilts.
5. Application of lime to infected soils has had no immediate or residual effect on the incidence of the disease.
6. Superphosphate seems to have some residual effect on the disease when applied to wilt affected soil.
7. Uspulun—dry or wet—when applied in large quantities to wilt affected soil, controls the incidence of wilt, but in small quantities it has no effect.
8. The incidence of wilt is influenced either by the relative positions of wilt affected and healthy soils in layers in a pot or by the proportion of wilt affected soil mixed with healthy soil.
9. It seems that the size of pots containing wilted soil in which plants are grown has some influence on the incidence of wilt.
10. *Fusarium* Strain A, commonly found in wilted plants, is present both in "wilted" and healthy soils in these Provinces.
11. Inoculation experiments have shown that this fungus is not parasitic.
12. Plants grown in healthy soil which is mixed with short lengths of stems of wilted cotton plants of the previous season become wilted, but this disease is also produced in plants, though to a much less extent, when the soil is mixed with healthy stems instead of diseased stems.
13. In healthy soil which is mixed with healthy cotton stems of the previous season and on which is cultured *Fusarium* Strain A, plants do not develop more wilt than in healthy soil mixed with uninoculated healthy cotton stems.
14. When single pieces of wilted cotton stems are planted in healthy soil along with each seed, there are no cases of wilt.

[1] Johnson, J., Slagg, C. M., and Murwin, H. F. Loc. cit.

15. Wilt can be developed in a healthy soil by non-pathogenic agencies, such as by treating it with heavy doses of organic manure or lime or with salts of aluminium, or by keeping the soil water-logged.
16. Typical wilt symptoms, macroscopic, microscopic and microchemical, can be produced in healthy plants growing in a " non-wilted " soil by injecting them with aluminium salt solutions ; *Fusarium* Strain A was found in those of the injected plants which were left in the soil for a long time after they had wilted.
17. Wilt is not induced in plants injected with water containing spores of *Fusarium* Strain A, or with filtrates of *Fusarium* Strain A, cultivated on Richard's solution.
18. Plants grown in a wilt affected soil, dry sterilized at 150°C. or 140°C., remain free from wilt, but if grown in " wilted " soil sterilized at 120°C. they are liable to wilt infection.
19. *Jowar* (*Sorghum*) seems to have some influence in controlling wilt.
20. Seedlings in very early stages of wilt infection, if transplanted to healthy soil, develop into healthy mature plants, though *Fusarium* Strain A has been found to be viable in these mature plants.
21. Plants grown in Knop's solution become wilt affected if aluminium salts are added to the solution, but remain healthy if only spores of *Fusarium* Strain A are added to it.
22. Staling experiments have shown that cotton plants are susceptible to the toxic effects of several *Fusaria* and other fungi.
23. It is suggested that aluminium salts are present in tissues of wilt affected plants, and wholly or partially fill the cell lumen.

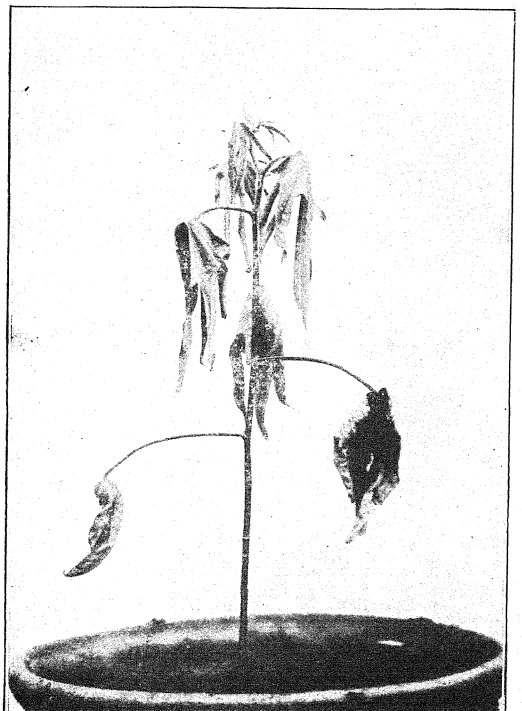


Fig. 1. A naturally wilt affected plant.

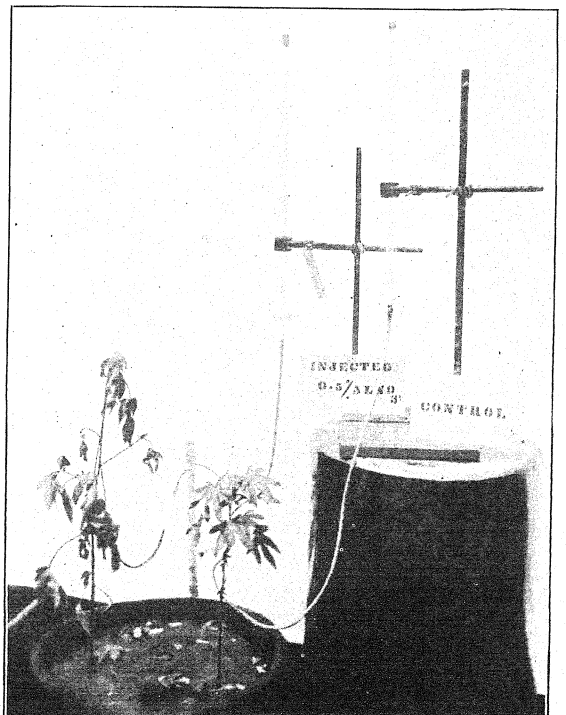
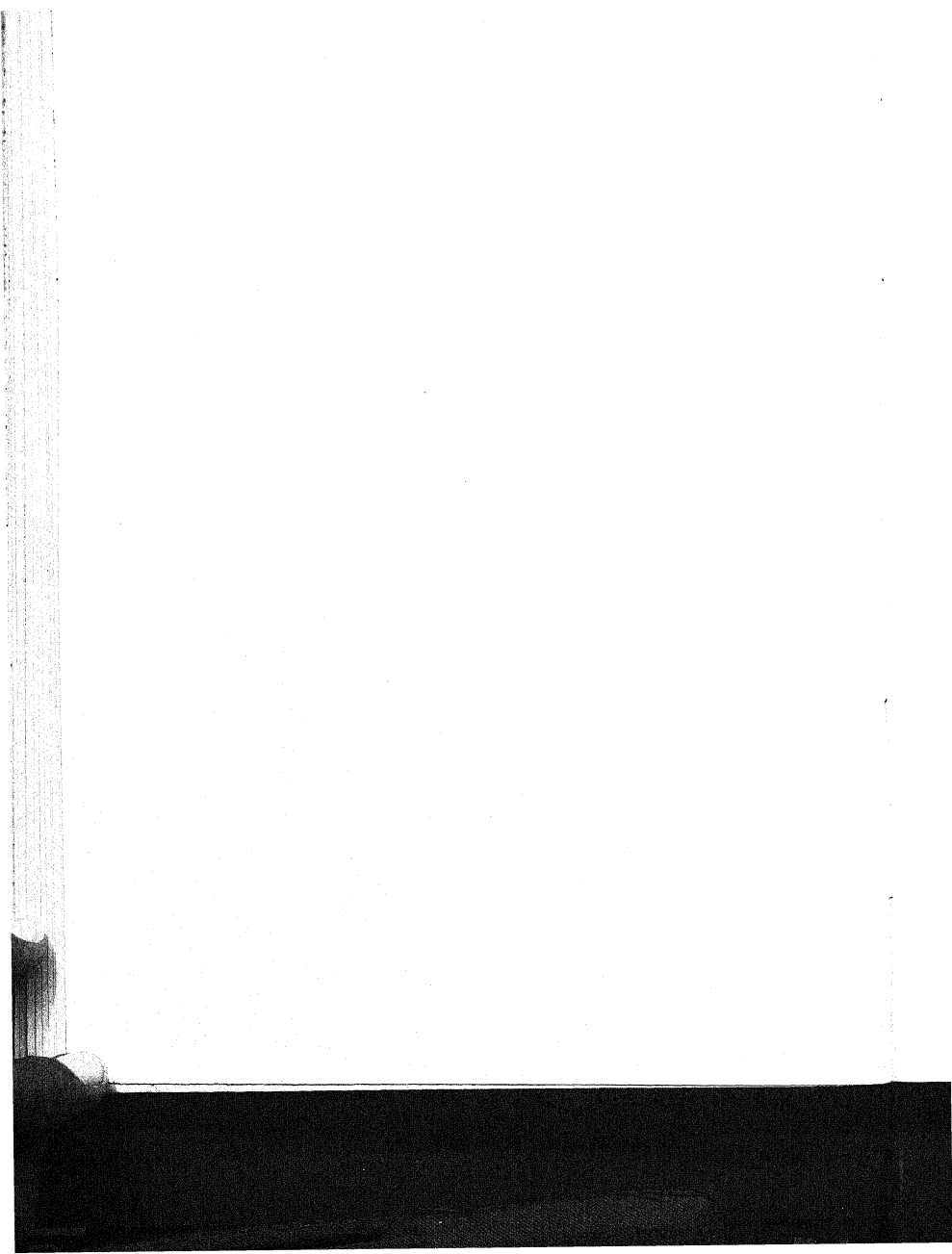


Fig. 2. A plant wilting as a result of injection with 0·5 per cent aluminium nitrate through a petiole. The plant on the right was injected with water through a petiole.

PLATE I.



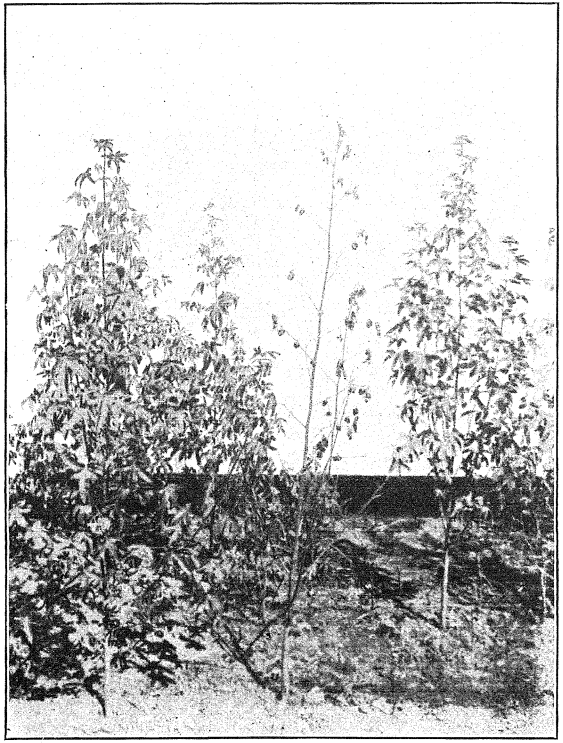


Fig. 1. Wilted and healthy plants in a field. No difference in branching or in growth of the diseased or healthy plants.

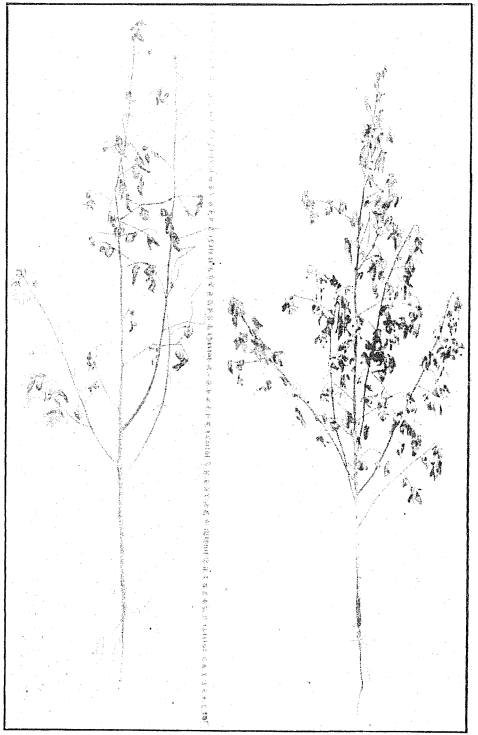


Fig. 2. Wilted and healthy plants from a field. No difference in growth of the stem or roots. (The leaves of the healthy plant lost turgidity after it was transplanted, hence the leaves are seen drooping in the plate.)

PLATE II.

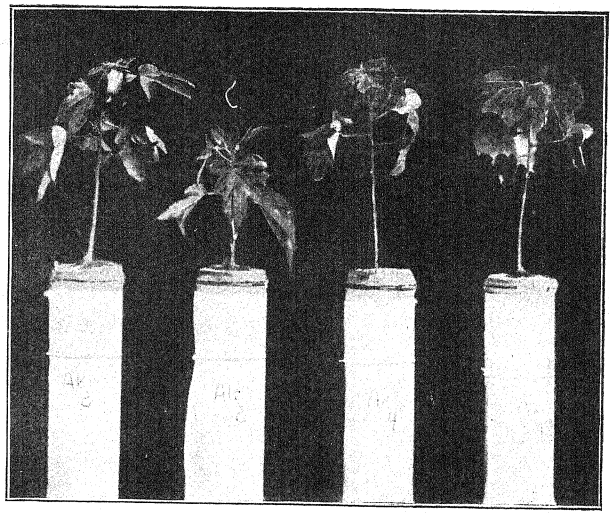


Fig. 1. Cotton plants—varieties AK_2 and AK_1—grown in half Knop's solution.

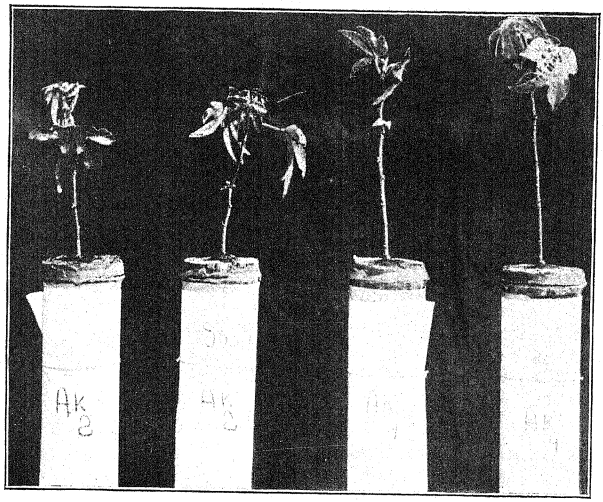


Fig. 2. Cotton plants—varieties AK_2 and AK_1—grown in equal parts of Knop's solution and N/900 aluminium nitrate.

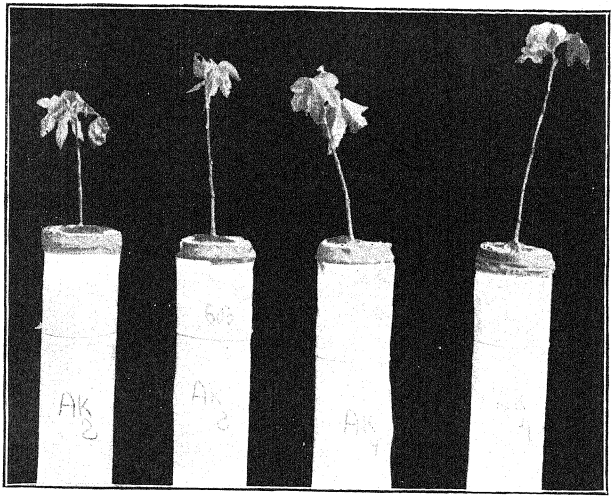


Fig. 1. Cotton plants—varieties AK_2 and AK_4—grown in equal parts of Knop's solution and N/600 aluminium nitrate.

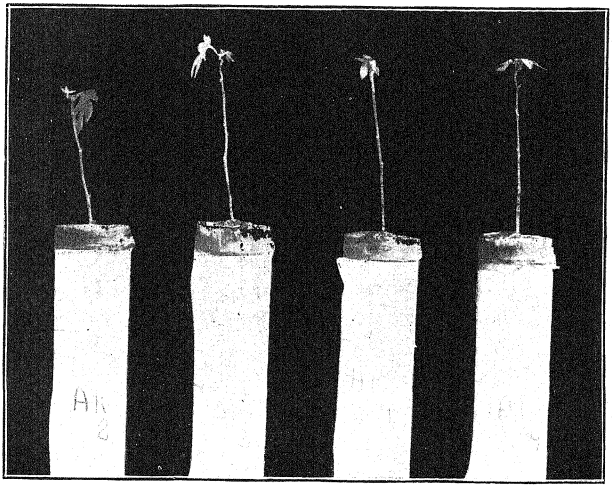


Fig. 2. Cotton plants—varieties AK_2 and AK_4—grown in equal parts of Knop's solution and N/300 aluminium nitrate.

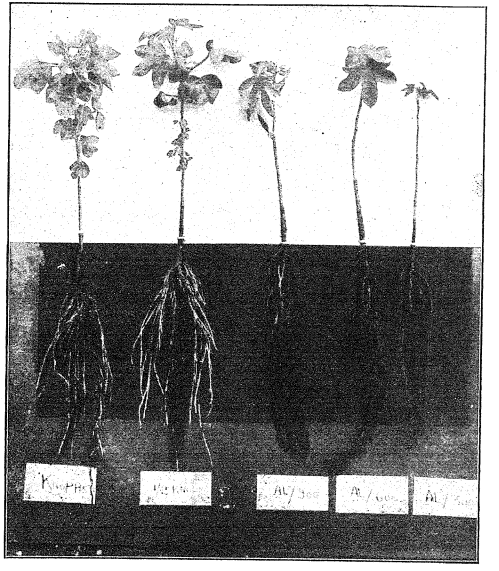


Fig. 1. Showing development of roots of plants, variety AK_2, grown in water cultures. From left to right, plant No. 1 grown in Knop's solution. No. 2 grown in half Knop's solution. No. 3 grown in equal parts of Knop's solution and N/900 aluminium nitrate. No. 4 grown in equal parts of Knop's solution and N/600 aluminium nitrate. No. 5 grown in equal parts of Knop's solution and N/300 aluminium nitrate.

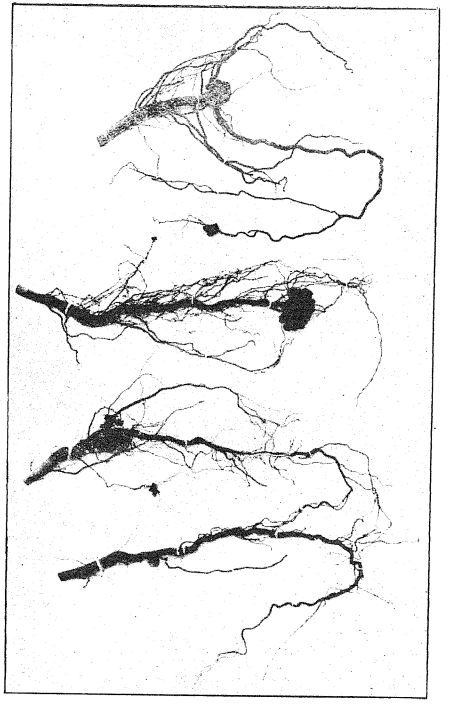


Fig. 2. Galls on roots of plants grown in healthy soil manured with *nim* cake.

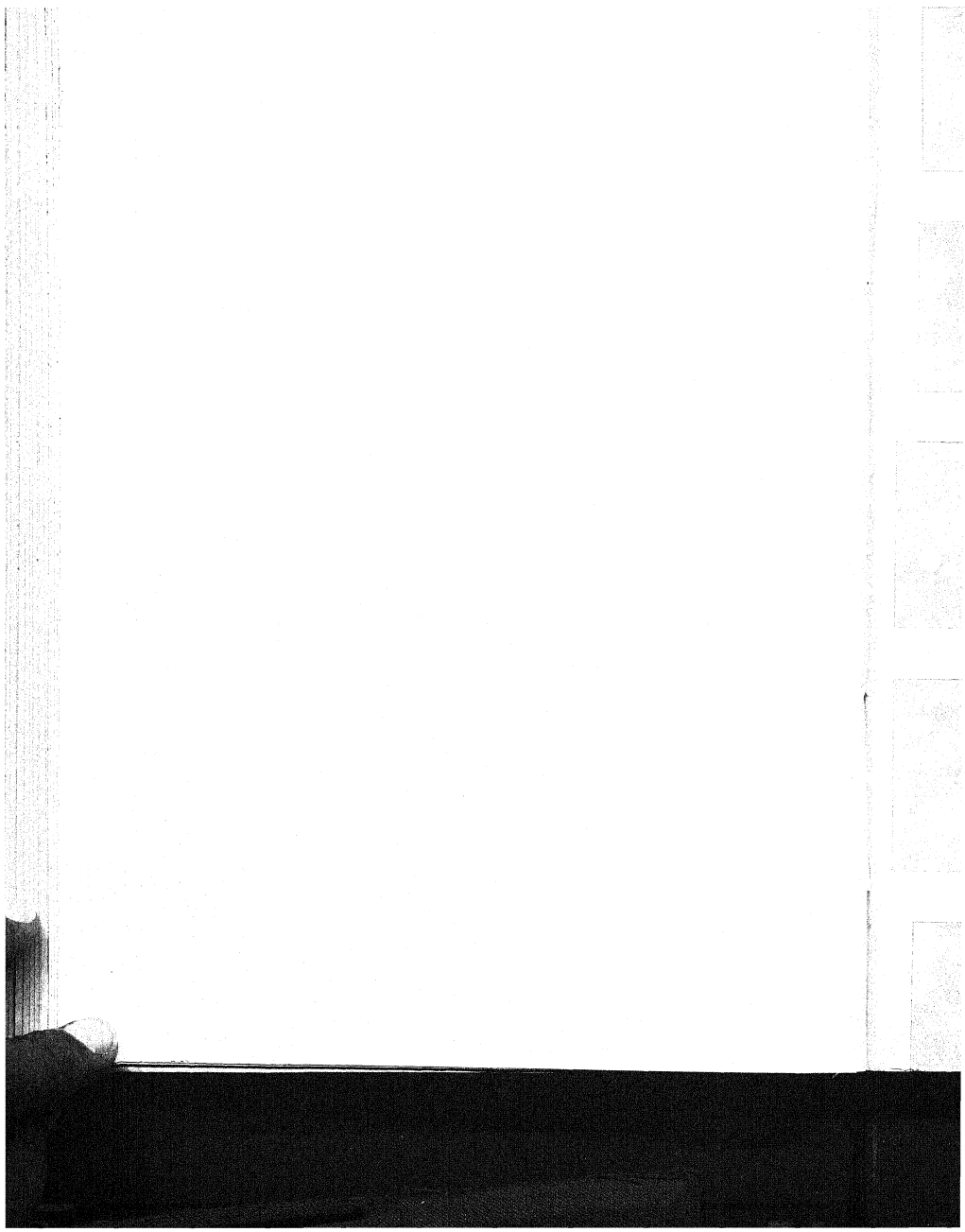


PLATE VI.

Fig. 1. Transverse section of a young healthy cotton plant, stained with iodine. × 180.

Fig. 2. Transverse section of a naturally wilted plant. × 180.

Fig. 3. Transverse section of a healthy plant at the end of the season. Note the partial or complete clogging of a few of the vessels. × 180.

Fig. 4. Transverse section of a plant wilting as a result of injection with aluminium nitrate. × 90.

Fig. 5. Transverse section of a wilting plant showing tyloses. × 370.

Figs. 6-8. Transverse sections of plants stained with iodine showing the presence of starch grains in medullary rays and in some xylem cells. × 370. Cf. Fig. 1.

Fig. 6. Naturally wilted plant.

Fig. 7. Healthy plant at the end of the season.

Fig. 8. Plant wilting as a result of injection with aluminium nitrate.

Fig. 9. Longitudinal section through a node of a wilting plant showing an aggregation of diseased cells. × 30.

Fig. 10. A transverse section of a root of a wilting plant through the point where a rootlet arises. Note the collection of diseased cells. × 30.

Fig. 11. Longitudinal section of a naturally wilted plant showing clogging of vessels. × 180.

Figs. 12-13. Transverse sections of a wilted plant and a healthy plant at the end of the season respectively showing presence of hyphæ in normal vessels. × 370.

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Fig. 9. Longitudinal section through a node of a wilting plant showing an aggregation of diseased cells. × 50.

Fig. 10. A transverse section of a root of a wilting plant through the point where a rootlet arises. Note the collection of diseased cells. × 50.

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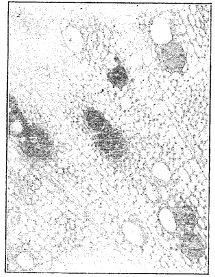


Fig. 2.

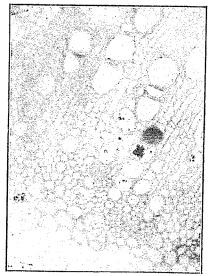


Fig. 3.

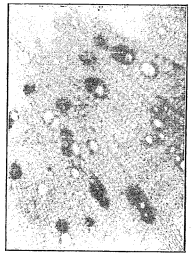


Fig. 4.

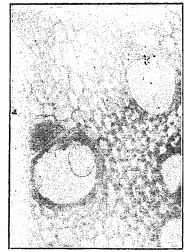


Fig. 5.

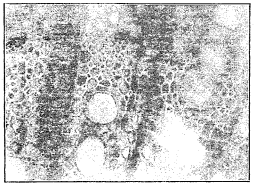


Fig. 6.

Fig. 7.

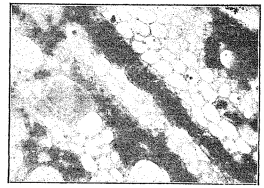


Fig. 8.

Fig. 9.

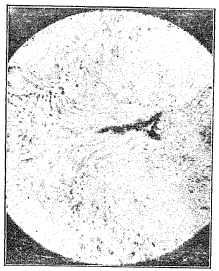


Fig. 10.

Fig. 11.

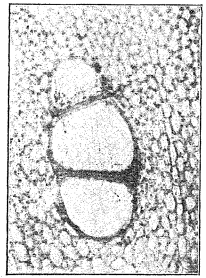


Fig. 12.

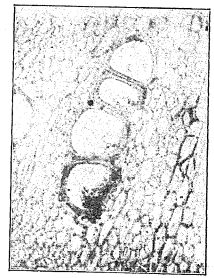
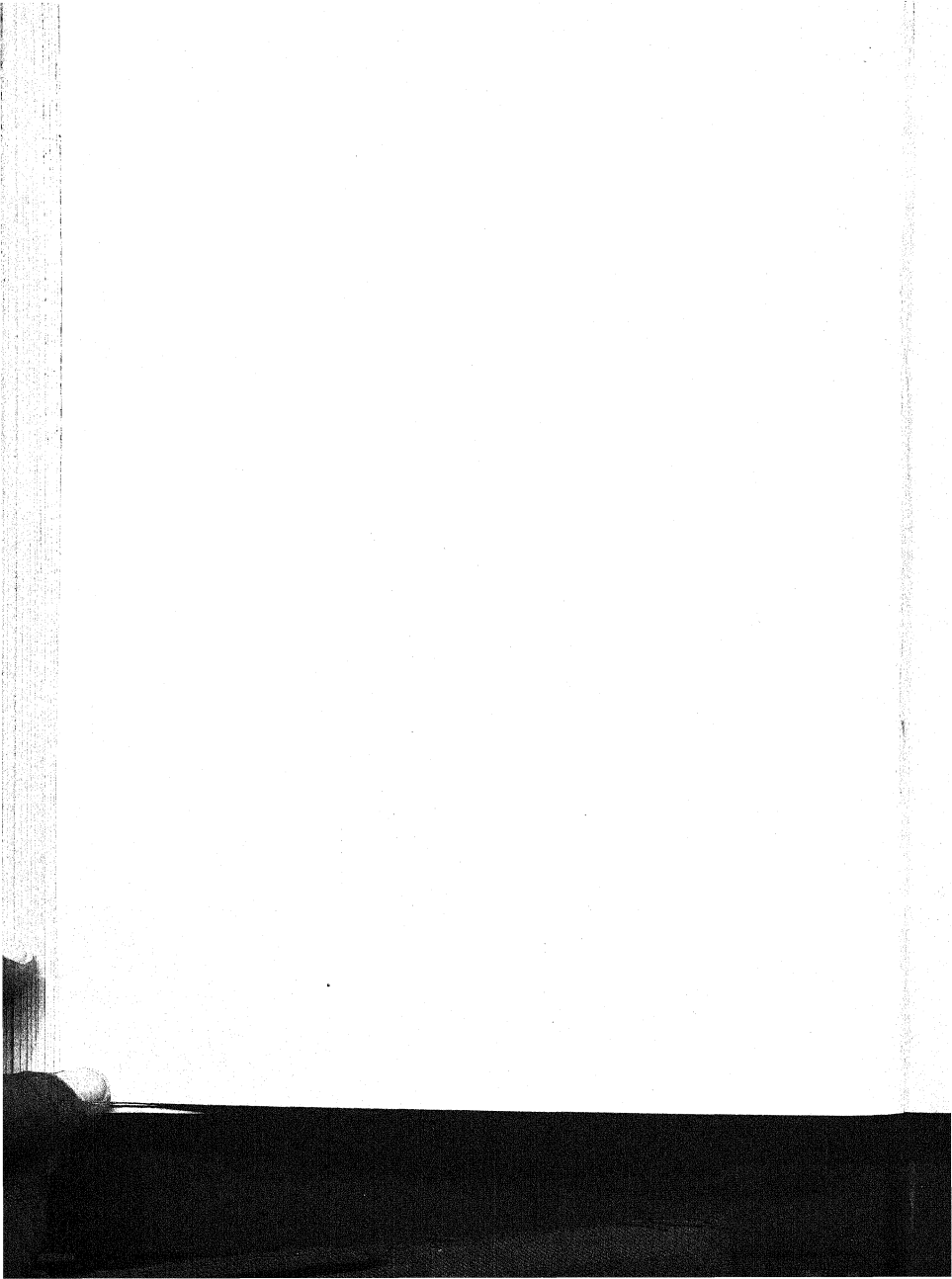


Fig. 13.



CONTENTS

STUDIES IN INHERITANCE IN COTTON.*

BY

MOHAMMAD AFZAL, B.Sc. (AGRI.), A.I.C.T.A.,

Senior Research Assistant, Cotton Research Laboratory, Lyallpur.

(Received for publication on 11th May 1929.)

INTRODUCTORY.

This paper deals with the history of a cross between *G. cernuum* and Burma Silky cotton, the latter being identified as *G. indicum* Gammie by Stock.[1] The work was carried out during the writer's residence at the Imperial College of Tropical Agriculture, Trinidad, and covers the history of the cross to the third generation. The mode of inheritance of the following characters was worked out :—

1. Leaf characters.
   - (*a*) Leaf shape.
     - (i) Leaf factor.
     - (ii) Leaf-lobe index.
     - (iii) Index of lowest sinus-breadth.
   - (*b*) Length of petiole.
2. Flower characters.
   - (*a*) Shape of bracts.
     - (i) Length of bracts.
     - (ii) Breadth of bracts.
   - (*b*) Corolla length.
3. Boll characters.
   - (*a*) Length of bolls.
   - (*b*) Width of bolls.
4. Lint and seed characters.
   - (*a*) Length of lint.
   - (*b*) Seed index.
   - (*c*) Lint index.

Though some of the characters were inherited in a simple manner, yet it was found that a very large number of them were very complicated. The frequency

* Accepted as a thesis for the Associateship of the Imperial College of Tropical Agriculture, Trinidad.

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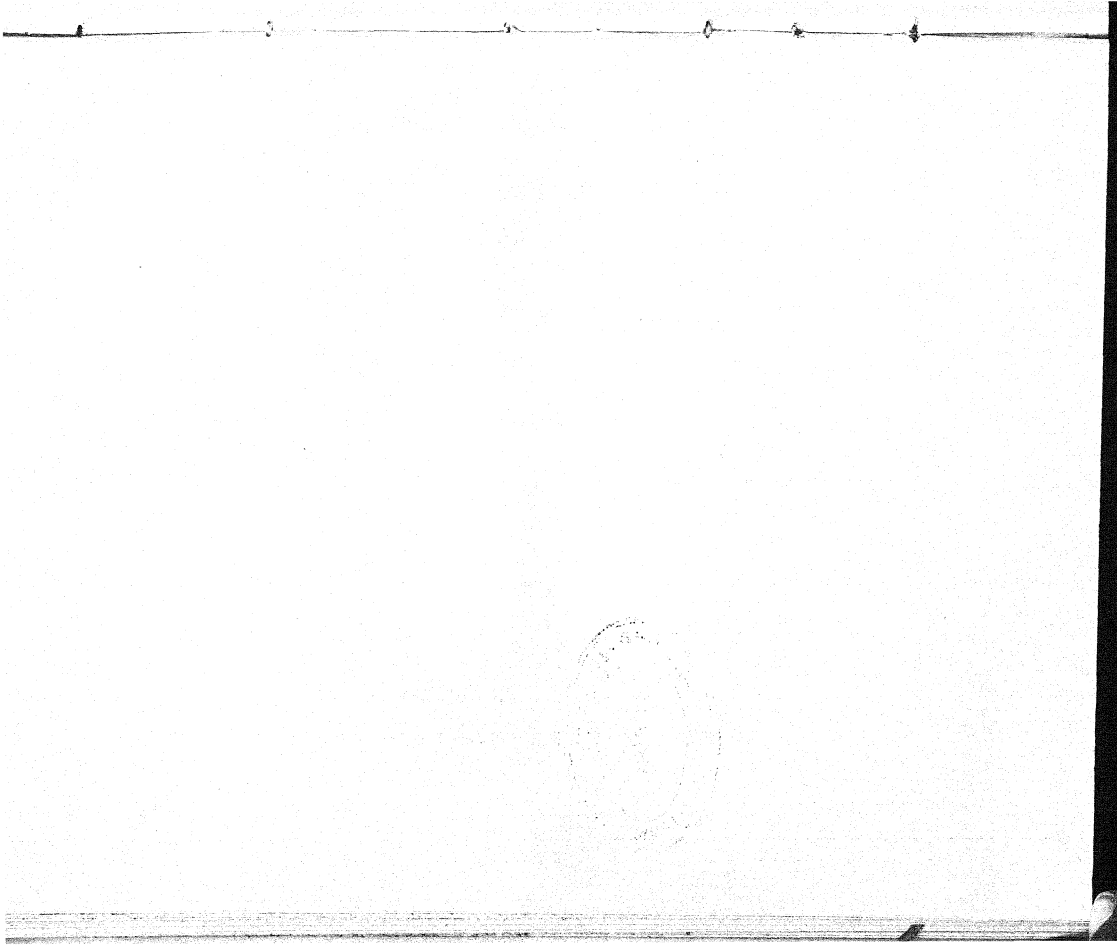

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curves for these characters were of the usual type obtained in the analysis of quantitative characters. A study of further generations might, possibly, have helped in their elucidation.

Wherever it was possible, ten measurements were taken for every character on each plant, but in no case were less than five measurements for each character taken. In the measurements of the leaves those borne directly on the main stem were taken, and in the case of bolls, measurements were confined to three-celled bolls for the sake of uniformity. Kottur has found that in Kumpta cotton the size of bolls varies with the type of branch on which they are borne. Patel, on the other hand, found no such difference in a strain of Broach Deshi cotton. The writer, however, measured the bolls borne on the primary sympodia only.

As will be noted in the sequel, the results of the F_3 generation are rather vitiated on account of the small number of plants in some of the families, but inspite of this, the general features of the tables are fairly clear.

1. Leaf characters.

(a) *Leaf shape.*

Interest in the mode of inheritance of leaf shape has been centered ever since the beginning of genetic experiments. The work so far done has extended over several genera and the following are only a few outstanding examples :—

Bateson[1] has quoted de Vries' experiments on *Chelinodium* where he found that laciniated type was recessive to the normal type of leaf.

Very recently Heijl and Uittien[2] confirmed de Vries' results and stated that the leaf form " deeply cut, often shortened and then digitate " is recessive to normal.

Shull[3] carried out extensive work on the four different biotypes or elementary species of *Busra bursa-pastoris* which he had himself isolated previously. He identified two genes, one which caused the elongation of the primary lobes and the other which caused the extension of the sinuses to the rachis and the presence of rounded secondary lobes in the distal axils of the primary lobes. He found that these two genes were independently inherited giving a 9 : 3 : 3 : 1 ratio in the F_2.

Peat[4] crossed two strains of *Ricinus communis* and found that the normal form of leaf was partially dominant to the deeply laciniated form. He gives the following F_2 figures :—

	Normal leaf	Laciniated leaf
Observed	364	126
Calculated (3 : 1)	367·5	122·5

[1] Bateson, W. (1909) *Mendel's Principles of Heredity.* Cam. Uni. Press.
[2] Heijl, M. W., and Uittien, H. (1926) *Genetica,* Deal VIII Afl. 3, en. 4.
[3] Shull, G. H. (1909) *Carnegie Inst., Washington, Pub. No. 112.*
[4] Peat, J. E. (1925) Genetic Studies in *Ricinus Communis,* L. Imp. Coll. Trop. Agri. Library.

He, therefore, came to the conclusion that the form of the leaf was controlled by a single pair of allelomorphic characters.

In cotton the work on the inheritance of leaf shape has been very extensive. The laciniated forms of cotton are found in both the New and the Old World groups and the origin of these forms has been a subject of much speculation. Some deeply laciniated forms have been definitely known to be mutations from the original stock of normal-leaved cottons. *G. Schottii* Watt is a wild or semi-wild form with deeply laciniated leaves occurring in Mexico,[1] and a laciniated type—'Okra cotton'—has originated spontaneously in commercial Upland varieties such as Acala. Occasional plants with deeply laciniated leaves have been met with in very isolated localities in the West Indian Islands such as Cassava type and Jamaica Xerophytic. This would also favour the view of their being true mutations.

The following is a brief resumé of the literature on the inheritance of leaf shape in cotton.

Fyson[2] was among the first to report his results of a cross between *G. neglectum* (narrow-lobed) and *G. herbaceum* (broad-lobed). The F_1 of this cross was narrow-lobed. From a study of the subsequent generations Fyson came to the conclusion that the narrow-lobed form of leaf was a simple dominant to the broad-lobed.

Leake[3],[4] devised a coefficient to represent the shape of the leaf which he called the leaf factor. He found that the pure strains fell into two groups, *viz.*, broad-lobed with leaf factor less than two and narrow-lobed with leaf factor greater than three. When these pure types were crossed the leaf factor of the F_1 was the arithmetic mean of the two parental types. The F_2 gave a trimodal distribution of the leaf factor and a close approximation to a 1 : 2 : 1 ratio, thus showing that the leaf factor was inherited as a simple Mendelian character. The parental forms extracted from the F_2 (with leaf factor less than two or greater than three) continued to breed true in the subsequent generations, while the heterozygotes (with leaf factor greater than two but less than three) continued to split in the normal way.

Kottur [5] in his studies of the leaf shape in a cross of *G. herbaceum* (Dharwar No. 1) and *G. neglectum* (*rosea*) raised some doubt as to the simple character of the leaf factor, and showed that the length and the breadth of the middle lobe were inherited independently of each other. In the F_2 he obtained the following porportion of the different types of leaves :—

Long broad-lobed leaves	8·3
Long narrow-lobed leaves	3·3
Short broad-lobed leaves	2·4
Short narrow-lobed leaves	1·0

[1] Watt, Sir George. (1907) *Wild and cultivated cotton plants of the World.*
[2] Fyson, P. F. (1908.) Some Experiments in the Hybridizing of Indian Cottons. *Mem. Dept. Agri. India, Bot. Ser.*, Vol. II, No. 6.
[3] Leake, H. M. (1911) *Jour. Genetics*, Vol. I, No. 3.
[4] Leake, H. M. (1911) *Proc. Roy. Soc. B. Ser.*, Vol. 83.
[5] Kottur, G. L. (1923) Studies in Inheritance in Cotton, I. History of a cross between *G. herbaceum* and *G. neglectum*. *Mem. Dept. Agri. India, Bot. Ser.*, Vol. XII, No. 3.

Shoemaker[1] crossed two Upland types of cotton, one the "Okra" mutant with deep cut narrow-lobed leaves and the other with normal leaves, and obtained an intermediate leaf shape in the F_1 and a close approximation to a 1 : 2 : 1 ratio in the F_2.

McLendon[2] crossed Sea Island with Upland and classified the F_2 by inspection only. He also got a close approximation to a 1 : 2 : 1 ratio in the F_2.

Balls[3] in his Upland-Egyptian crosses used the expression S/L to indicate the extent of laciniation, S being the distance of the upper sinus from the petiole and L the length of the midrib. He found that the deeply laciniated form was dominant in the F_1. The segregation in the F_2, however, did not conform to any definite ratio.

Kearney[4] in his Holdon-Pima cross used the same expression as Balls to indicate the depth of laciniation and found that the deeply laciniated form was dominant in the F_1. The F_2, however, failed to show any regular segregation.

Peebles and Kearney [5] crossed two strains of Acala cotton; one with normal leaves and the other with 'Okra-leaves.' The F_1 was intermediate in leaf shape and the F_2 gave a very close approximation to a 1 : 2 : 1 ratio. The authors point out that the segregation was very clear cut and the three forms were readily recognizable in the field.

In the present cross the writer studied the inheritance of leaf shape according to the following three modes of determination :—

(i) Leaf factor $\frac{a-b}{c}$

(ii) Leaf-lobe index . . . . . $\frac{a}{b}$

NOTE.—Balls and Kearney have used the expression $\frac{b}{a}$, but the writer has used the converse.

(iii) Index of lowest sinus-breadth . . . . . $\frac{a}{d}$

(Fig. 1).

(i) *Leaf factor.* The results obtained by the writer are in full accord with those of Leake. The leaf factor of the broad-lobed parent (Burma Silky) was less than two and that of the narrow-lobed parent (*G. cernuum*) greater than three. It is to be regretted, however, that the frequency array of the leaf factors of the F_1 plants is not available, as the number of these plants was limited; but it was noted that the leaf factor of those grown was, in each case, greater than two but less than three. Great divergence in the leaf factors of the F_2 plants was obtained and the frequency array is given in Table I.

[1] Shoemaker, D. N. (1909) *Amer. Bree. Assoc. Rep.* Vol. V. Quoted by Kearney.
[2] McLendon, C. A. (1912) *Ga. Expt. Sta. Bull.* No. 99. Quoted by Kearney.
[3] Balls, W. L. (1912) The *Cotton Plant in Egypt.* Macmillan & Co., London.
[4] Kearney, T. H. (1923) *U. S. Dept. Agri. Bull.* No. 1164.
[5] Peebles, R. H., and Kearney, T. H. (1928) *Jour. Heredity*, Vol. XIX, No. 5.

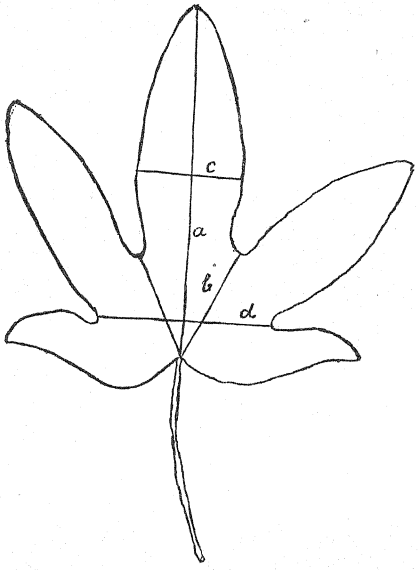


FIG. 1. DIAGRAMMATIC REPRESENTATION OF COTTON LEAF SHOWING THE LOCATION OF MEASUREMENTS.

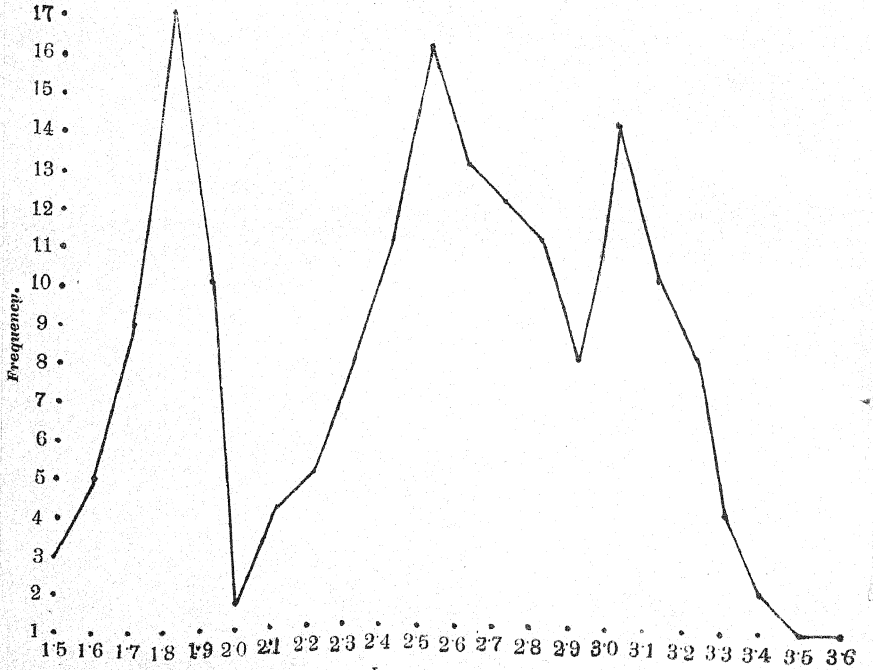
TABLE I.

Leaf factor	Frequency
1·5	3
1·6	5
1·7	9
1·8	17
1·9	10
2·0	2
2·1	4
2·2	5
2·3	8
2·4	11
2·5	16
2·6	13
2·7	12
2·8	11
2·9	8

TABLE I—*contd.*

Leaf factor	Frequency
3·0	14
3·1	10
3·2	8
3·3	4
3·4	2
3·5	1
3·6	1
TOTAL	174

The frequency curve drawn from the above Table is shown in Fig. 2.



LEAF FACTOR.
FIG. 2.

This frequency curve could obviously be dissected into three classes. There is no real point of division, however, between the two higher classes and the curve shows that these overlap. This division is, therefore, to some extent arbitrary though useful as an indication of the real state of affairs. An analysis of the figures in Table I gives the following results :—

TABLE II.

	Narrow-lobed (Cernuum type)	Intermediate type	Broad-lobed (Indicum type)
Observed	44	80	39
Calculated (1 : 2 : 1 ratio) . . .	41	82	41

NOTE.—In analysing the data presented in Table I, the figures of the "minimum frequency classes" have been excluded from the calculations as pointed out by Hoshino[1]. This procedure has been followed in all the analyses of the figures presented in the present paper.

It will be seen from Table II that the observed figures agree very closely with the calculated and thus fall in line with Leake's hypothesis.

The results of the F_3 generation are presented in Table III.

TABLE III.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF LEAF FACTORS OF F_3 FAMILIES									Mean
		1·4	1·7	2·0	2·3	2·6	2·9	3·2	3·5	3·8	
1-4 .	1·5	4	27	7	..	..	..	..	..	..	1·7
2-5 .	2·0	..	..	..	2	1	..	..	..	..	2·4
2-6 .	2·0	5	6	5	16	14	4	..	..	..	2·2
1-9 .	2·1	..	2	..	..	3	2	..	..	..	2·4
2-4 .	2·1	..	1	..	7	3	..	..	..	..	2·3
2-2 .	2·2	..	..	3	3	2	1	..	..	..	2·3
1-7 .	2·4	..	5	1	2	4	3	4	..	..	2·5
2-1 .	2·4	..	..	1	1	..	1	1	..	..	2·6
1-6 .	2·5	1	1	1	..	1	5	..	4	1	2·8
1-8 .	2·5	..	1	1	1	2	4	2	2	..	2·8
1-2 .	2·6	..	..	..	..	8	4	1	..	..	2·7
1-1 .	3·0	..	..	..	..	..	3	11	17	1	3·3
1-3 .	3·1	..	..	..	..	..	1	1	..	..	3·0

From the above Table it will be seen that in the F_3 the average leaf factor of each family approximates to that of the parent. The narrow- and the broad-lobed plants of the F_2 generation bred pure in the F_3, while the intermediate plants

[1] Hoshino, Y. (1915) *Jour. Coll. Agri. Sapporo., Japan*, Vol. VI, Part IX.

split up and gave a large number of broad- or narrow-lobed offspring according as their leaf factors approximated to the broad- or the narrow-lobed types.

(ii) *Leaf-lobe index.* Balls[1] and Kearney[2] in their Upland-Egyptian crosses found that whereas the leaf-lobe index was nearly the same in the F_1 as that of the Egyptian parent, the results of the F_2 generation could not be analysed.

In the cross under investigation the writer has, however, found evidence of monohybrid segregation. The leaf-lobe index of *G. cernuum* was 5·0 and that of the Burma Silky cotton 2·6. The F_1 generation had a mean leaf-lobe index of 2·9. The frequency array of the F_2 generation is given in the subjoined table.

TABLE IV.

Leaf-lobe index	Frequency
2·3	7
2·6	33
2·9	9
3·2	16
3·5	18
3·8	21
4·1	31
4·4	21
4·7	8
5·0	5
5·3	2
5·6	3
TOTAL	174

The above frequency array will give a bimodal curve (Fig. 3).

Here the shallow-lobed class (leaf-lobe index less than 2·9) can be readily recognized, but the intermediate and the deep narrow-lobed classes are not easily discernable. The frequency array can, therefore, be split into only two classes, the results being given in Table V.

TABLE V.

	Leaf-lobe index greater than 2·9	Leaf-lobe index less than 2·9
Observed	125	40
Calculated (3 : 1)	123·75	41·25

NOTE. The frequency at class 2·9 (Table IV) is here omitted, being the minimum frequency.

The very close approximation of the recorded to the calculated figures is quite patent.

[1] Balls, W. L. loc. cit.
[2] Kearney, T. H. loc. cit.

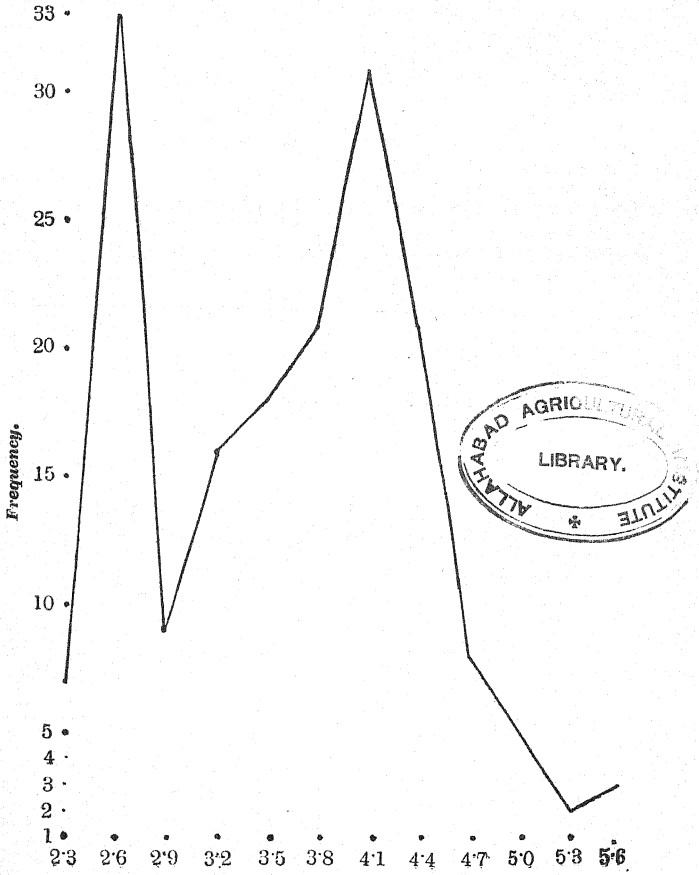


FIG. 3.

Further support of the hypothesis that the leaf-lobe index is inherited in a monohybrid ratio is found in the very high correlation which exists between the figures of the leaf-lobe index and those of the leaf factor, the coefficient of correlation being $+0{\cdot}900 \pm 0{\cdot}009$. It is to be argued that since the leaf factor gives a monohybrid segregation (page 81) and since the F_2 values of the leaf factor bear a very high positive correlation with the corresponding values of the leaf-lobe index, the latter must also manifest a similar type of segregation.

The results of the F_3 generation are in full accord with the above hypothesis. While the extracted shallow broad-lobed and the deep narrow-lobed plants bred true in the F_3 generation, the intermediate plants split up and gave a large number of shallow broad- or deep narrow-lobed plants according as their leaf-lobe index approximated to the broad- or the narrow-lobed plants. Table VI below gives the results of the F_3 generation.

TABLE VI.

Family No. (F_3)	Parental value (F_2)	Frequency arrays of leaf-lobe index of F_3 families								
		2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	Mean
1-4 . .	2·2	34	4	..	..	..	..	..	..	2·5
1-9 . .	2·7	1	1	3	2	..	..	..	..	3·4
2-5 . .	2·7	..	1	2	..	..	..	..	..	3·3
2-6 . .	2·7	8	8	18	13	3	..	..	..	3·4
2-4 . .	2·9	2	1	6	2	..	..	..	..	3·4
1-8 . .	3·0	2	..	4	3	3	1	..	..	3·8
2-2 . .	3·1	3	..	1	5	..	..	..	..	3·5
1-2 . .	3·3	..	..	6	5	2	..	..	..	3·8
2-1 . .	3·3	1	..	1	1	1	..	..	..	3·6
1-7 . .	3·4	5	1	2	4	3	3	..	1	3·8
1-6 . .	3·5	2	1	2	3	1	3	..	3	4·3
1-1 . .	4·0	..	..	..	..	7	17	6	1	5·1
1-3 . .	4·1	..	..	..	..	1	1	..	..	4·7

The slight tendency towards parent-offspring correlation suggests that minor genes are also operating.

(iii) *Index of lowest sinus-breadth.* This index is inherited in very much the same way as the leaf-lobe index. Since the breadth of the lowest sinus (*d*. in Fig. 1) bears a very high positive correlation with the distance of the upper sinus from the petiole (*b*) and also with the breadth of the middle lobe (*c*), the shape of the leaf will be represented as follows :—

1. A high value of the index of lowest sinus-breadth will represent a deeply cut narrow-lobed leaf.
2. A low value of this index will indicate a shallow broad-lobed leaf.

The results obtained in the present cross are as follows :—

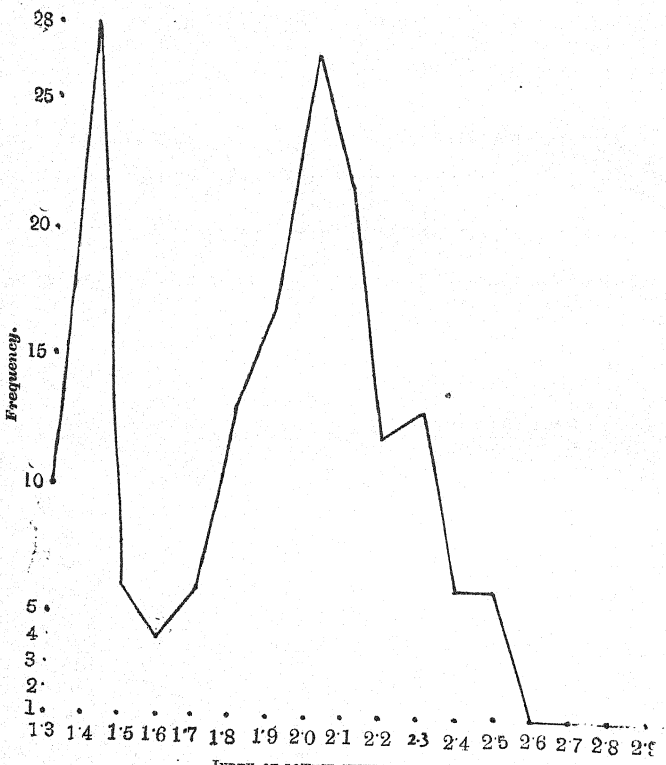
Index of lowest sinus-breadth of *G. cernuum*	3·0
,, ,, ,, ,, of Burma Silky	1·4
,, ,, ,, ,, of F_1	1·8

The frequency array of the index of lowest sinus-breadth of the F_2 generation is given in the following table.

TABLE VII.

Index of lowest sinus-breadth	Frequency
1·3	10
1·4	28
1·5	6
1·6	4
1·7	6
1·8	13
1·9	17
2·0	27
2·1	22
2·2	12
2·3	13
2·4	6
2·5	6
2·6	1
2·7	1
2·8	1
2·9	1
TOTAL	174

The curve drawn from the above table is shown in Fig. 4.



INDEX OF LOWEST SINUS-BREADTH.

FIG. 4.

The above frequency curve is bimodal and can be dissected into only two classes as shown in Table VIII.

TABLE VIII.

	Index value above 1·6	Index value below 1·6
Observed	126	44
Calculated (3 : 1)	127·5	42·5

NOTE.—The frequency at class 1·6 is the 'Minimum frequency' and is therefore not calculated.

As will be seen from the above table, the calculated figures agree closely with the observed figures proving thereby that the index in question is controlled by a single pair of allelomorphs.

A study of the F_3 generation revealed a slightly greater degree of variation in this character than was met with in the F_2 generation. In discussing the inheritance of size characters, East [1] says :—" In generations succeeding the F_2 the variability of any family may be less but not greater than the variability of the population from which it came," it being assumed that the number of plants in both generations concerned is large enough to include all genotypes. The slightly greater range of variation of the F_3 in the present case may, possibly, be due to the greater vigour of growth as it was grown on better soil and under more congenial conditions than the F_2. The subjoined table gives the results of the F_3 generation.

TABLE IX.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF THE INDEX OF LOWEST SINUS-BREADTH OF THE F_3 FAMILIES.								
		1·3	1·6	1·9	2·2	2·5	2·8	3·1	3·4	Mean
1-4 . .	1·2	18	20	..	..	..	..	..	..	1·4
1-9 . .	1·6	2	..	..	1	3	1	..	..	2·1
2-6 . .	1·6	3	8	17	13	9	..	..	..	2·0
1-6 . .	1·7	..	1	1	3	3	3	2	1	2·5
2-4 . .	1·7	..	1	3	6	1	..	..	..	2·1
2-5 . .	1·7	..	..	1	1	1	..	..	..	2·2
1-2 . .	1·8	..	..	5	8	..	..	..	..	2·1
2-2 . .	1·8	..	3	..	2	3	1	..	..	2·2

[1] East, E. M. (1916) *Genetics*, Vol. I. Quoted by Kearney.

TABLE IX—*contd.*

Family No. (F_3)	Parental value (F_2)	Frequency arrays of the index of lowest sinus-breadth of the F_3 families.								Mean
		1·3	1·6	1·9	2·2	2·5	2·8	3·1	3·4	
1-7 . .	1·9	5	..	..	7	1	5	1	..	2·2
1-8 . .	1·9	..	2	..	..	6	3	..	2	2·6
2-1 . .	2·0	..	1	..	2	1	..	..	..	2·1
1-1 . .	2·2	..	..	..	..	4	8	15	5	3·0
1-3 . .	2·3	..	..	..	..	..	1	1	..	2·9

Comparing Tables VII and IX, we find that the plants with values near the extremes bred true, while the intermediate plants split up in regular order, thus justifying the assumption that the character under study is, in all probability, controlled by a single pair of allelomorphs. The presence of minor genes is also indicated by the parent-offspring correlation.

In reviewing the mode of inheritance of the degree of laciniation measured in three different ways, the results of the F_2 and F_3 generations seem to indicate that a single pair of main genes is involved. It has also been shown that minor genes are operating. The progenies of the plants near the extremes of the F_2 distribution were far less variable than those of the plants around the mean. This agrees with Freeman's findings[1]:—"If the differences in the means of F_3 cultures.....are due to genetic causes, one would expect the intermediate cultures to be more variable than the extremes, thus assuming that the extreme cultures are more nearly homozygous than those which are intermediate."

It is also interesting that in each of the three cases mentioned above, the segregation in the F_2 generation of plants approaching the Burma Silky parent was very sharp. The curves shown in Plates II, III and IV show this very clearly. Moreover in the first two cases (Leaf factor and Leaf-lobe index) the curves trail off beyond the extreme variation of the *cernuum* parent. The probable explanation is that the modifying genes which have already been indicated to be present are only effective in the presence of the main gene supplied by the *cernuum* parent, but have no effect in the presence of the gene supplied by the Burma Silky parent.

(*b*) *Length of petiole.*

The mode of inheritance of petiole length seems not to have been studied and, so far as the writer knows, no results have been reported. In the present cross the

[1] Freeman, G. F. (1919) *Genetics* Vol. IV. Quoted by Kearney.

inheritance of petiole length was studied, but the results are at present inexplicable and a large number of genes seems to be involved.

The petioles of the F_1 plants were longer than those of either parent, probably due to heterosis. The following figures demonstrate this :—

Average petiole length of Burma Silky	5·1 cm.
" " " of *G. cernuum*	5·5 cm.
" " " of F_1	6·6 cm.

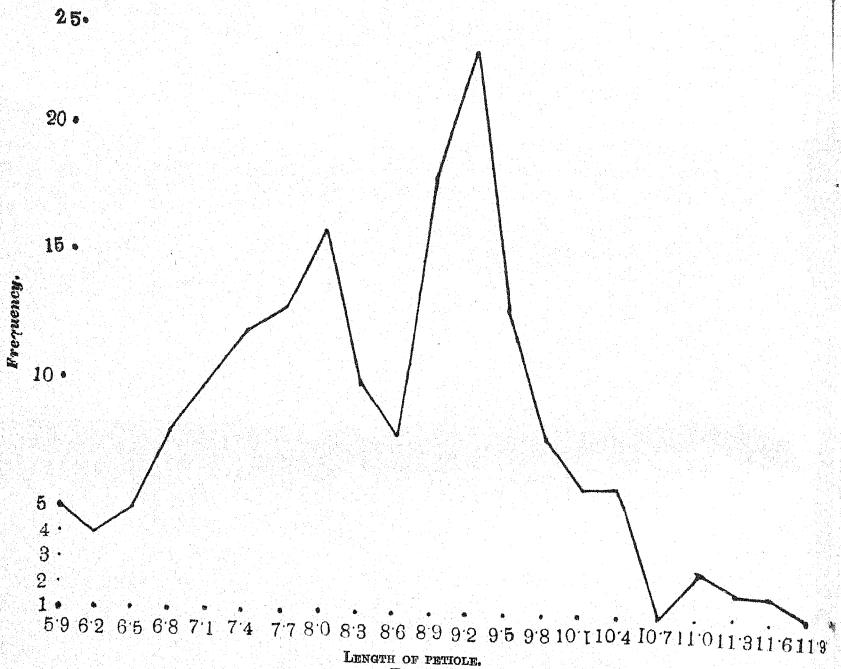
The frequency array of petiole length of the F_2 plants is given in the following Table.

TABLE X.

Length of petiole (cm.)	Frequency
5·9	5
6·2	4
6·5	5
6·8	8
7·1	10
7·4	12
7·7	13
8·0	16
8·3	10
8·6	8
8·9	18
9·2	23
9·5	13
9·8	8
10·1	6
10·4	6
10·7	1
11·0	3
11·3	2
11·6	2
11·9	1
TOTAL	174

NOTE.—Three plants had petioles less than 5·8 cm. long but are included in class 5·9.

The frequency curve drawn from the above figures is shown in Fig. 5.



LENGTH OF PETIOLE.

FIG. 5.

The curve shown in Fig. 5 can apparently be dissected into two at class 8·6. This will give the following results :—

TABLE XI.

Petiole length above 8·6	Petiole length below 8·6
83	83

The analysis of the figures as shown above is not quite legitimate as due to heterosis, which has been shown to occur, the segregation of the genes for length is masked by the segregation of the growth factors. For similar reasons the results of the F_3 generation shown in Table XII cannot be analysed.

TABLE XII.

Family No. F_3	Parental value F_2	Frequency arrays of petiole length of F_3 families																								Mean
		5·0	5·3	5·6	5·9	6·2	6·5	6·8	7·1	7·4	7·7	8·0	8·3	8·6	8·9	9·2	9·5	9·8	10·1	10·4	10·7	11·0	11·3	11·6		
1-9 . .	8·6	..	..	1	..	1	..	2	1	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..		6·8
2-1 . .	8·9	..	..	..	..	..	1	2	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..		7·0
1-8 . .	9·0	..	..	..	..	1	..	3	1	2	3	2	..	1	..	..	..	..	..	..	..	..	..	..		7·4
2-6 . .	9·2	1	..	..	5	..	4	6	4	8	6	9	2	2	2	..	1	..	..	..	..	..	..	..		7·4
2-5 . .	9·3	..	..	..	..	2	1	..	..	1	..	3	1	1	..	..	..	..	..	..	..	..	..	..		7·4
1-4 . .	9·4	..	..	..	3	2	8	9	7	5	2	2	..	..	..	..	..	..	..	..	..	..	..	..		6·8
1-1 . .	9·6	..	..	..	..	..	..	..	..	..	..	..	4	1	4	2	3	7	1	4	..	3	1	2		9·7
1-6 . .	9·6	..	1	1	1	..	2	2	..	1	3	1	1	..	..	..	..	..	..	..	..	..	..	..		6·7
1-7 . .	9·7	..	..	1	1	3	2	2	6	1	2	..	..	..	..	..	..	..	..	..	..	..	..	..		6·8
2-4 . .	9·7	1	1	..	2	2	6	..	..	3	..	3	1	1	..	..	..	..	..	..	..	..	..	..		6·8
1-3 . .	10·0	..	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..		8·0
2-2 . .	10·0	..	..	..	..	2	1	..	..	1	..	1	1	1	..	..	..	..	..	..	..	..	..	..		7·3
1-2 . .	10·6	..	..	..	1	..	1	4	..	3	2	2	..	..	..	..	..	..	..	..	..	..	..	..		7·2

The scatter of the figures in the above table does not suggest any analysis whatever. Family No. 1-1 is very interesting as here the longest petioles are met with.

It is, however, interesting to note that the length of petiole bears a fairly high positive correlation to the length of the midrib, the coefficient of correlation being $+0{\cdot}6995 \pm 0{\cdot}026$.

2. Flower characters.

(*a*) *Shape of bracts.*

Balls[1] from his studies in Egypt concluded that the narrow form of bract was dominant over the broad; but the exact mode of inheritance in the F_2 and subsequent generations was not worked out.

Kearney[2] in his Holdon-Pima cross found that the mean bract length of the F_1 exceeded that of the either parent and that in the F_2 the frequency curve of bract length was unimodal, the range of variation exceeding that of the either parent.

The results obtained by the writer, while differing in minor details, agree in the main issues with those of Balls and Kearney.

(*i*) *Length of bracts.* In the Holdon-Pima cross Kearney found, as stated above, that the mean bract length of the F_1 was greater than that of either parent, but in the present cross the mean bract length of the F_1 was almost the arithmetic mean of those of the two parents, as the following figures will show.

Mean bract length of *G. cernuum*	5·5 cm.
" " " of Burma Silky	3·6 cm.
" " " of F_1	4·3 cm.

The frequency array of bract lengths of the F_2 plants, shown in Table XIII below, is rather interesting in that it shows a decided preponderance of plants with long bracts; in fact there was found only one plant with really short bracts.

Table XIII.

Length of bracts (cm.)	Frequency
3·6	1
4·2	3
4·3	3
4·4	10
4·5	11
4·6	15
4·7	16
4·8	24
4·9	16
5·0	22
5·1	14
5·2	8
5·3	9
5·4	5
5·5	2
5·6	3
5·7	2
5·8	1
Total	165

[1] Balls, W. L. loc. cit.
[2] Kearney, T. H. loc. cit.

The curve plotted from the above figures is shown in Fig. 6.

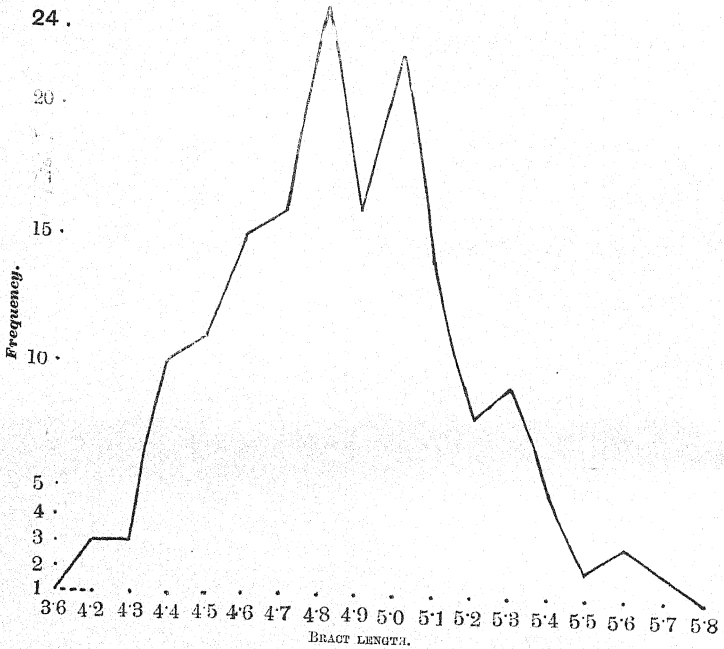


Fig. 6.

The above curve is slightly bimodal and is inexplicable ; so too is the great preponderance of plants with intermediate and long bracts unaccountable.

It is to be regretted that F_3 progenies from plants with very long, as well as those with very short bracts could not be grown. The parental bract lengths of the thirteen F_3 families grown ranged from 4·0 to 5·0 cm. Therefore the conclusions drawn here are tentative.

It is rather curious that in all the thirteen F_3 families very little splitting was noticed inspite of the fact that these were all derived from the middle portion

of the F_2 curve. The results of the F_3 generation are presented in Table XIV below.

TABLE XIV.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF BRACT LENGTH OF F_3 FAMILIES							Mean
		3·5	3·8	4·1	4·4	4·7	5·0	5·3	
2-6 .	4·0	7	23	19	6	..	..	..	3·97
2-5 .	4·1	..	2	2	..	..	..	..	3·95
1-7 .	4·2	..	5	7	5	..	..	..	4·10
1-9 .	4·2	..	6	2	..	..	..	..	3·88
2-1 .	4·2	..	..	..	3	1	..	..	4·48
2-2 .	4·2	..	3	4	1	..	..	..	4·03
1-2 .	4·3	..	..	3	9	2	..	..	4·38
1-4 .	4·4	..	1	9	22	5	1	..	4·37
1-6 .	4·4	..	..	3	9	1	..	..	4·35
1-8 .	4·5	..	..	4	4	3	1	..	4·43
2-4 .	4·5	..	..	1	5	3	..	..	4·47
1-1 .	4·8	..	..	4	11	11	4	2	4·60
1-3 .	5·0	..	..	..	..	1	1	..	4·85

The parent-offspring correlation calculated from the above table is $+0{\cdot}962 \pm 0{\cdot}0034$. This very high coefficient of heredity is a further proof that in point of bract length all the F_3 families have bred practically true to the parental value and there occurred but little splitting. This is contrary to expectations as ordinarily the splitting must have been quite noticeable. This may be due to modifying genes.

(*ii*) *Breadth of bracts.* With regard to the breadth of the bracts Balls[1] found in his crosses that narrow bract was dominant over the broad, but in the cross under investigation the reverse was found to be the case, as will be seen below.

Mean bract breadth of *G. cernuum* . . . . . 3·7 cm.
" " " of Burma Silky . . . . . 3·4 cm.
" " " of F_1 . . . . . 3·6 cm.

The F_2 generation was very variable in this character ; the variation extended beyond the extremes of either parent. The frequency array of the F_2 population is shown below :

[1] Balls, W. L. loc. cit.

TABLE XV.

Breadth of bracts (cm.)	Frequency
2·8	1
2·9	1
3·0	2
3·1	1
3·2	2
3·3	4
3·4	10
3·5	24
3·6	27
3·7	22
3·8	24
3·9	13
4·0	16
4·1	6
4·2	8
4·3	3
4·4	0
4·5	0
4·6	1
TOTAL	165

The curve drawn from the above frequency array, as shown in Fig. 7. is multimodal but very probably with a larger number of plants would become unimodal. The curve can not be easily explained, the only suggestion being that several genes are involved.

The data obtained from the F_3 generation further confirms this view as the splitting observed was very indefinite. The results of the F_3 generation are given in Table XVI.

TABLE XVI.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF BRACT BREADTH OF F_3 FAMILIES.							Mean
		2·6	2·9	3·2	3·5	3·8	4·1	4·4	
2-5	3·4	..	1	3	..	..	..	..	3·13
2-6	3·4	..	12	29	12	2	..	..	3·22
1-2	3·5	..	1	4	7	2	..	..	3·41
1-7	3·5	1	3	7	5	1	..	..	3·24
1-9	3·5	..	..	5	3	..	..	..	3·31
1-4	3·7	..	3	10	17	6	1	1	3·46
2-2	3·7	..	..	3	5	..	..	..	3·39
1-3	3·8	..	..	..	..	1	..	1	4·10
1-6	3·8	..	..	1	6	6	..	..	3·61
2-1	3·8	..	..	..	4	..	..	..	3·50
2-4	3·8	..	..	1	3	5	..	..	3·63
1-1	4·0	..	..	..	10	11	11	..	3·81
1-8	4·2	..	..	..	5	5	2	..	3·73

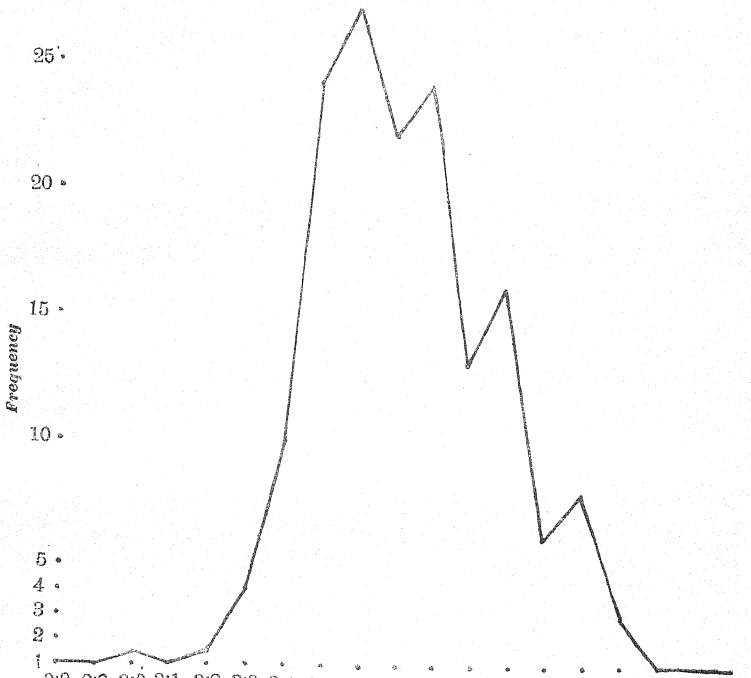


Fig. 7.

The coefficient of heredity calculated from Table XVI is $+0{\cdot}626 \pm 0{\cdot}035$. This somewhat low value of the coefficient, as compared with that obtained in the case of bract-length, is due to the splitting which has taken place in some of the families.

A fairly strong correlation was observed between the length and breadth of bracts ; the coefficient of correlation was $+0{\cdot}616 \pm 0{\cdot}032$.

(*b*) *Length of corolla.*

According to Balls[1] the length of petals is inherited in a 3 : 1 ratio, though he says the curve of the frequency array is "deformed by fluctuations, both autogenous and ordinary".

On the other hand, Kearney[2] in his Holdon-Pima cross found that the petal-length in the F_1 was greater than that of either parent, and the F_2 gave a unimodal curve with mean petal-length very near to that of the Pima parent. He gives the following figures.

	Mean petal-length (mm.)
Holdon	35·1±1·10
Pima	56·8±0·70
F_1	61·0±0·34
F_2	52·6±0·37

Kottur[3] in his cross between Dharwar No. 1 and Rosea found that although in the F_1 the long petal was dominant, in the F_2 a proportion of 1 long : 2·8 short was obtained. He has not suggested any explanation.

In the writer's cross the two parents were very distinct as regards their petal-length, the mean petal-length of Burma Silky being 3·0 cm. and that of *cernuum* 5·1 cm. The F_1 was intermediate having a mean petal-length 3·8 cm. The F_2, however, showed a decided preponderance of plants with short petals; the frequency array is shown in the following table.

TABLE XVII.

Length of petals (cm.)	Frequency
2·6	1
2·7	1
2·8	3
2·9	4
3·0	6
3·1	14
3·2	19
3·3	35
3·4	26
3·5	15
3·6	21
3·7	10
3·8	8
3·9	2
4·0	1
TOTAL	166

It will be noted that the range of variation extends beyond the extreme of the short parent but does not reach even to the mean of the long parent.

The curve of the frequency array is shown in Fig. 8. It is practically unimodal and very difficult to explain.

[1] Balls, W. L. loc. cit.
[2] Kearney, T. H. loc. cit.
[3] Kottur, G. L. loc. cit.

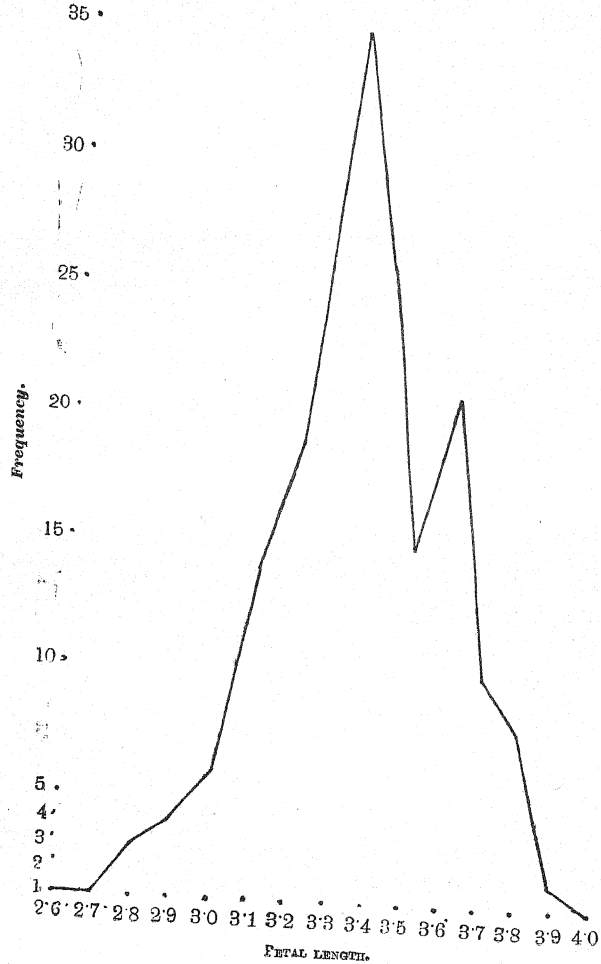

35
30
25
20
15
10
5
4
3
2
1
Frequency.
2·6 2·7 2·8 2·9 3·0 3·1 3·2 3·3 3·4 3·5 3·6 3·7 3·8 3·9 4·0
Petal length.

Fig. 8.

It may, however, be interesting to compare the results of Kearney and Kottur with those obtained in the present cross.

	Mean petal-length (mm.)					
	Holdon	Pima	Rosea	Dharwar No. 1	Cernuum	Burma Silky
	35·1	56·8	18·7	26·7	51·0	30·0
	68·0		26·3		37·0	
F_1	52·6		19·0		34·0	
F_2						

Fig. 9 shows the curves plotted from the figures of which the above are the means. It may be noted from the above that—

1. Kearney's results on New World cottons stand in marked contrast to those of Kottur and the writer on the Old World cottons.
2. The length of petal is dominant in the Holdon-Pima cross, but this is not the case in the Dharwar No. 1—Rosea and Burma Silky—*cernuum* crosses.
3. The exact mode of inheritance of the length of petals is not yet quite understood.

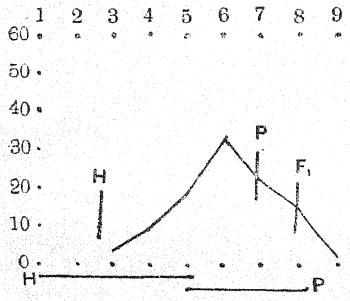
The results of the F_3 generation are given in Table XVIII.

Table XVIII.

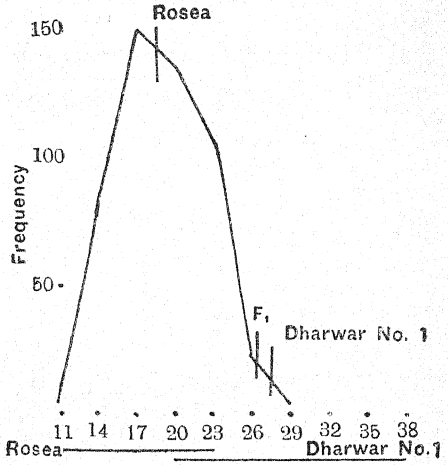
Family No. (F_3)	Parental value (F_2)	Frequency arrays of corolla length of F_3 families												
		2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0	Mean
2-2	2·9	1	1	3	3	..	..	..	..	..	..	..	..	3·1
2-5	2·9	..	..	1	1	..	1	1	..	..	..	..	..	3·30
2-8	2·9	1	8	9	8	15	8	3	3	..	..	..	..	3·24
1-6	3·0	..	..	..	2	2	5	1	1	1	1	..	..	3·43
1-1	3·1	..	..	1	..	5	7	6	7	4	2	..	..	3·50
1-7	3·1	1	..	2	1	6	6	2	..	..	..	..	..	3·30
1-9	3·1	..	1	..	1	..	..	4	..	..	1	..	1	3·50
1-4	3·2	..	..	1	1	3	14	9	7	..	2	..	1	3·48
1-2	3·4	..	..	..	..	1	2	3	3	3	..	2	..	3·59
2-4	3·5	..	..	..	..	..	1	1	1	2	1	1	1	3·70
1-3	3·7	..	..	..	..	..	..	..	..	..	..	1	1	3·95
1-8	3·7	..	..	..	..	..	4	1	2	3	..	2	..	3·60
2-1	3·7	..	..	..	..	..	..	1	..	..	2	1	..	3·75

It is apparent from the above table that most of the families show splitting in a very indefinite manner, and the mode of inheritance of petal-length remains unexplained.

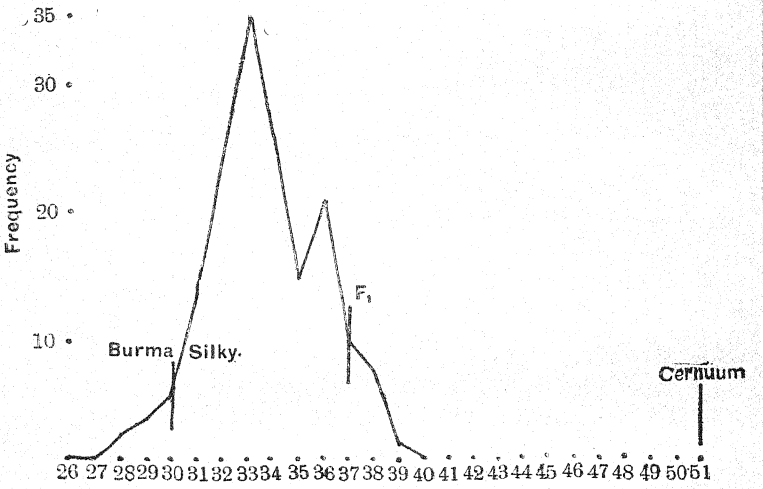
It is interesting to note that no plant with petals more than 4·0 cm. long was found either in the F_2 or the F_3, though the mean petal-length of one of the parents (*G. cernuum*) was 5·1 cm.



Corolla-length distribution in the F_2 of the Holdon-Pima Cross (after Kearney)



Corolla-length distribution in the F_2 of the Dharwar No. 1—Rosea Cross (From Kottur's Figures)



Corolla-length distribution in the F_2 of the Burma-Silky-Cernuum Cross

FIG. 9.

c

A fairly marked correlation was observed between the length of petals and the length of bracts, the coefficient of correlation was $+0{\cdot}50 \pm 0{\cdot}04$.

No linkage whatever was found to exist between the shape of the leaves and the length of corolla. The *cernuum* parent was narrow-lobed with long corolla, while the Burma Silky parent had broad-lobed leaves and short corolla. In the F_2 long and short corollas occurred altogether irrespective of the shape of the leaves. There was no linkage between the length of the leaves and the length of the petals either.

3. Boll characters.

Balls[1] has shown that in all the Upland-Egyptian crosses studied by him the mode of inheritance of boll index (width relative to length) was very complex.

Kearney[2] also records a similar complexity of boll index in his Holdon-Pima cross and suggests that several genes are involved.

Patel[3] has studied the fluctuations in the size and length of bolls in several pure strains of Broach Deshi cotton, and he shows that these characters remain relatively constant from season to season. He has not studied the mode of inheritance.

The data obtained by the writer on the length and width of bolls in this cross admits of no analysis and the only inference drawn is that both these characters are highly complex in their inheritance and that several genes are involved.

(*a*) *Length of bolls.*

In this character the two parents bore a remarkable contrast. The average length of boll of *G. cernuum* was 6·1 cm. and that of Burma Silky 3·3 cm.

The average length of bolls of the F_1 plants was 4·0 cm. In the F_2 it was noticed that, inspite of a fairly large number of plants, the very long type of boll (*cernuum* type) was not recovered. The frequency array of the length of bolls of the F_2 generation is shown in the following Table :—

Table XIX.

Length of boll (cm.)	Frequency
3·4	2
3·5	1
3·6	6
3·7	11
3·8	8
3·9	13
4·0	15
4·1	21
4·2	23
4·3	18
4·4	17
4·5	11
4·6	15
4·7	4
4·8	7
4·9	4
Total	176

[1] Balls, W. L. loc. cit.
[2] Kearney, T. H. loc. cit.
[3] Patel, M. L. (1924) *Mem. Dept. Agri. India, Bot. Ser.*, Vol. XII, No. 5.

The slight multimodality of the curve (Fig. 10) drawn from the above frequency array is probably due to minor fluctuations, and the frequency array remains unexplained.

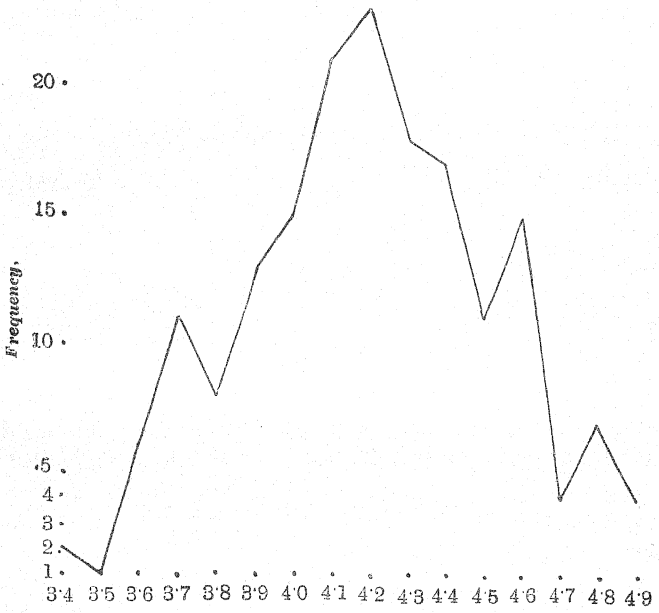


Fig. 10.

The figures of the F_3 generation further confirmed the idea of several genes being involved in the inheritance of the length of boll. In Table XX are shown the F_2 frequency arrays of thirteen families.

TABLE XX.

Family No. (F_3)	Parental value (F_2)	Frequency arrays of boll-length of F_3 families						
		3·4	3·7	4·0	4·3	4·6	4·9	Mean
2-6 . .	3·5	3	19	27	5	..	..	3·9
2-2 . .	3·6	..	2	6	1	..	..	3·9
1-9 . .	3·7	1	1	2	1	..	..	3·9
2-1 . .	3·8	..	..	3	..	..	..	4·0
2-5 . .	3·8	..	..	2	1	1	..	4·2
1-1 . .	3·9	..	2	2	16	11	1	4·4
1-7 . .	3·9	..	3	11	1	..	..	3·8
1-4 . .	4·0	..	..	4	25	11	..	4·4
1-6 . .	4·0	..	..	2	7	1	..	4·3
1-8 . .	4·0	..	2	2	6	1	..	4·2
1-2 . .	4·1	..	..	1	11	2	..	4·3
2-4 . .	4·3	..	..	3	4	3	1	4·4
1-3 . .	4·4	..	..	..	..	..	2	4·9

The F_3 families were drawn from different places in the F_2 frequency curve, but they fail to show much difference in splitting.

Another point worthy of note is that in no case the length of bolls exceeded 4·9 cm.—the extreme value of the F_2 frequency.

(*b*) *Width of bolls.*

The width of bolls is inherited in much the same way as the length, and probably here too several genes are involved. Following figures are available :—

Average width of bolls of Burma Silky	2·7 cm.
„ „ „ of *G. cernuum*	3·4 cm.
„ „ „ of F_1	2·8 cm.

These figures apparently show the dominance of the small type of boll, but the subsequent generations proved this hypothesis to be untenable. The frequency array of the width of bolls of the F_2 generation is shown in Table XXI.

TABLE XXI.

Width of bolls (cm.)	Frequency
2·5	1
2·6	10
2·7	33
2·8	66
2·9	45
3·0	16
3·1	3
3·2	1
3·3	1
TOTAL	176

The curve plotted out from the above frequency array is shown in Fig. 11. The curve is steep and symmetrical and is quite inexplicable.

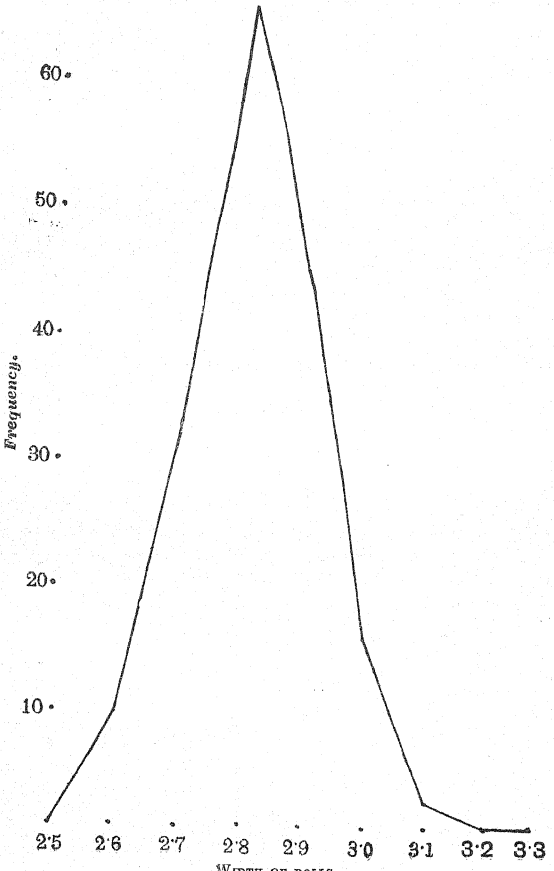

60
50
40
30
20
10
Frequency.
2·5
2·6
2·7
2·8
2·9
3·0
3·1
3·2
3·3
Width of bolls.

Fig. 11.

D

The frequency arrays of the F_3 families are shown in Table XXII.

TABLE XXII.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF BOLL-WIDTH OF F_3 FAMILIES							
		2·6	2·7	2·8	2·9	3·0	3·1	3·2	Mean
1·6 .	2·3	..	..	5	3	2	..	..	2·9
2·2 .	2·6	..	1	6	2	..	..	..	2·8
2·5 .	2·6	..	..	1	3	..	..	..	2·9
1·1 .	2·7	..	1	4	11	11	5	..	2·9
1·4 .	2·7	..	1	11	21	6	1	..	2·9
1·7 .	2·7	..	3	5	6	1	..	..	2·8
1·9 .	2·7	..	..	4	1	..	..	..	2·8
1·2 .	2·8	..	..	2	5	5	1	1	3·0
1·3 .	2·8	..	1	3	6	..	..	1	2·9
2·1 .	2·8	..	..	1	1	1	..	..	2·9
2·6 .	2·8	2	10	22	19	1	..	..	2·8
1·3 .	2·9	..	..	..	..	..	2	..	3·1
2·4 .	3·0	..	..	..	6	4	1	..	3·0

Though the F_3 families are drawn from different points on the F_2 frequency curve, they split irregularly and do not help in explaining the mode of inheritance of the width of bolls.

In summarizing the mode of inheritance of the two characters of bolls described above, the following points are worthy of note :—

1. The mode of inheritance was very complex and probably several genes were involved in each case.
2. The plants with big bolls were not recovered either in the F_2 or the F_3.

4. LINT AND SEED CHARACTERS.

(*a*) *Length of lint.*

From the economic point of view, the length of lint is one of the most important characters of the cotton plant ; and the problems connected with the improving

of the length and fineness of lint and also the combining of these qualities with other desirable characters have always been subjects of great interest.

Fyson [1] came to the conclusion that " .....length and fineness of lint were completely dominant over short and rough woolly nature."

Balls[2] in his inter Egyptian and Upland-Egyptian crosses was able to extract pure parental forms from the subsequent generations, though, he says, some of the intermediate forms also bred true. He concluded that length of lint was inherited in a 3 : 1 ratio.

Kearney [3] in his Holdon-Pima cross found that mean lint-length of the F_1 generation was very near to that of the long parent (Pima), but the F_2 generation gave a unimodal curve.

Kottur[4] confirmed the results obtained by Fyson. He also detected a linkage between the colour and length of lint, brown colour being associated with short lint.

The results obtained in the present cross seem to agree with those of Kearney. The figures will be found below :—

Average lint-length of *G. cernuum*	20·8 mm.
,, ,, of Burma Silky	29·4 mm.
,, ,, of F_1	28·1 mm.

The frequency array of the F_2 generation is given below.

TABLE XXIII.

Length of lint (mm.)	Frequency
18·5	6
19·5	2
20·5	52
21·5	39
22·5	24
23·5	26
24·5	15
25·5	15
26·5	3
27·5	2
TOTAL	184

[1] Fyson, P. F. loc. cit.
[2] Balls, W. L. loc. cit.
[3] Kearney, T. H. loc. cit.
[4] Kottur, G. L. loc. cit.

The curve drawn from the above figures is shown in Fig. 12. The curve is unimodal and suggests that several genes are involved.

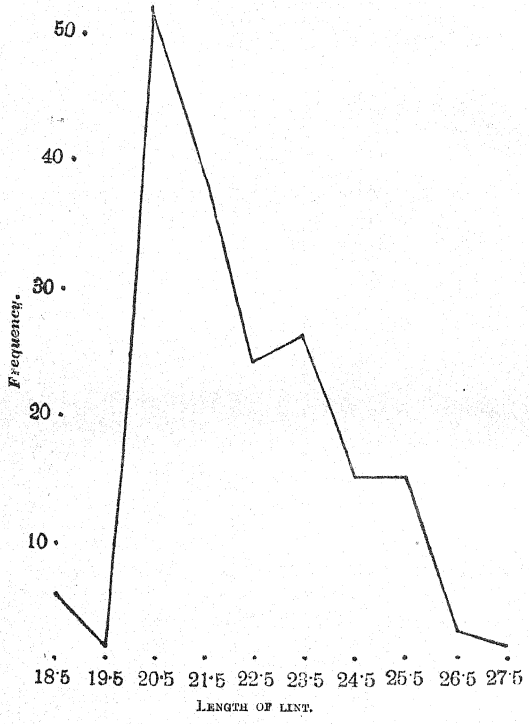


Fig. 12.

The results of the F_3 generation are presented in the subjoined table.

TABLE XXIV.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF LINT-LENGTH OF F_3 FAMILIES												Mean
		16·5	17·5	18·5	19·5	20·5	21·5	22·5	23·5	24·5	25·5	26·5	27·5	
2-5 . .	21·5	..	..	..	..	2	2	1	..	..	..	..	..	21·3
1-8 . .	23·0	1	..	..	..	7	4	..	..	1	1	..	..	21·1
1-9 . .	23·5	1	..	..	1	4	3	3	..	..	..	..	..	20·9
2-1 . .	24·2	..	..	..	..	2	..	1	2	..	..	..	..	22·1
2-6 . .	24·5	..	..	..	1	5	2	9	16	10	11	1	1	23·6
1-2 . .	25·0	..	..	..	..	2	4	5	..	1	1	..	1	22·6
2-4 . .	25·0	..	..	..	..	..	..	2	..	1	3	..	1	24·8
1-3 . .	25·5	..	..	..	..	..	..	1	..	..	1	..	..	24·0
1-4 . .	25·8	..	..	..	2	11	6	8	5	5	3	..	..	22·2
1-7 . .	27·0	..	..	..	..	2	..	1	6	2	5	..	..	23·8
1-1 . .	29·0	..	..	..	..	..	..	2	2	11	15	2	..	24·9
2-2 . .	29·1	..	..	..	..	..	..	1	1	..	5	..	1	25·2
1-6 . .	30·0	..	..	..	..	..	..	1	5	5	3	..	1	24·4

All the F_3 families would give unimodal frequency curves. The figures do not throw much light on the mode of inheritance of the length of lint. The above table confirms the view that several genes probably control the inheritance of lint-length in the cross under investigation.

(b) Seed weight.

Balls[1] summarizes the behaviour of mean seed weight in several crosses by saying that "beneath all the complexity involved by fluctuations, by autogenous fluctuations and correlations, there existed in all these hybrids a straight forward segregation of seed-size controlled by a single allelomorphic pair of factors in every case."

The results obtained by the writer cannot, however, be explained on the assumption of simple inheritance and seem to indicate that several genes are at work.

The mean seed index (weight of 100 seeds) of Burma Silky was 57 mg. and that of *G. cernuum* 92 mg. The F_1 generation had a mean seed index of 72 mg. It will be seen from the above figures that the "intensification" of this character, as noticed by Balls[1], did not occur in the present case.

[1] Balls, W. L. loc. cit.

The frequency distribution of seed index in the F_2 generation is given below.

TABLE XXV.

Seed index (mg.)	Frequency
47	3
52	8
57	28
62	35
67	39
72	39
77	21
82	5
87	4
92	2
TOTAL	184

The curve drawn from the above frequency array as shown in Fig. 13 is unimodal and the only probable explanation is that several genes control the expression of this character.

The results of the F_3 generation are presented in the following Table.

TABLE XXVI.

Family No. (F_3)	Parental value (F_2)	FREQUENCY ARRAYS OF SEED INDEX IN F_3 FAMILIES											
		4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	Mean
2-6	4·8	2	1	1	1	7	10	19	9	3	1	1	6·9
1-6	7·3	..	..	..	1	..	5	4	3	2	..	..	6·9
2-1	7·4	..	..	..	..	..	1	1	1	1	1	..	7·5
1-7	8·0	..	..	2	..	2	6	4	1	..	..	..	6·4
1-9	8·0	1	..	1	2	1	1	3	1	1	..	1	6·5
2-5	8·0	..	..	1	..	..	1	2	1	..	..	..	6·6
1-3	8·4	..	..	..	1	..	..	..	1	..	..	..	6·5
1-8	8·7	..	1	1	1	..	4	3	1	..	2	1	6·8
2-4	9·2	..	..	..	..	..	1	2	1	1	2	..	6·4
2-2	9·3	..	..	..	..	..	..	3	3	1	1	..	7·5
1-2	9·7	..	..	..	..	..	4	5	1	3	1	..	7·2
1-1	9·9	..	..	..	..	3	6	10	12	1	..	..	7·0
1-4	10·1	..	..	..	..	5	2	11	12	7	2	1	7·3

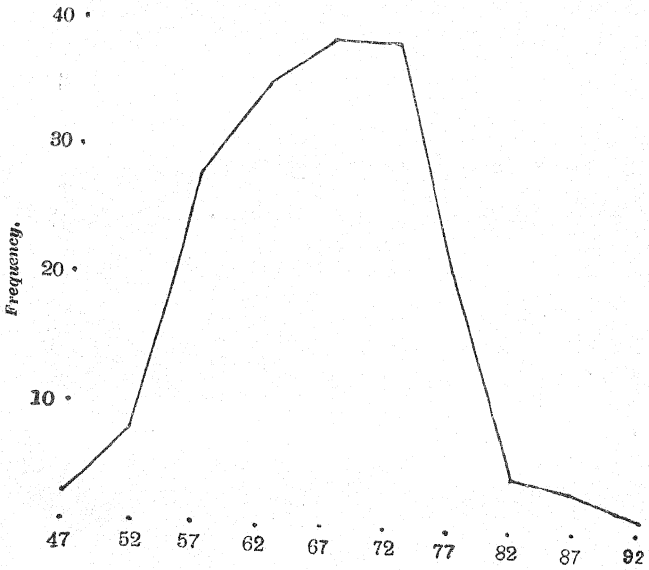


FIG. 13.

An examination of the above table would show that several genes are responsible for seed index, thus confirming the conclusion reached at after the examination of the F_2 figures. There is very little difference in splitting observed in the families 1-8, 1-9 and 2-6 though their parental values differ widely. Family 1-4 has the highest parental value and, contrary to expectation, shows fairly pronounced splitting. The presence of the *plus* and *minus* modifying genes, probably several in number, is fairly clear.

(c) *Lint index.*

The importance of this character in commercial cotton breeding has long been recognized. Harland[1] says: "……out of the morphological characters bearing on yield, weight of lint per seed is the most important by virtue of the high positive correlations which it exhibits both with the weight of lint per boll and the weight of lint per acre." The same author has elsewhere[2] discussed at full length the desirability of taking lint index (weight of lint on 100 seeds) rather than the ginning percentage as the basis of selection.

Kearney[3] obtained a unimodal curve for this character in the F_2 generation of his Holdon-Pima cross. The range of variation in the F_2 far exceeded that of either parent especially on the lower side of the curve.

The results obtained in the present cross are of the same type as those obtained by Kearney. The figures will be found below.

Lint index of *G. cernuum*	61 mg.
„ „ of Burma Silky	37 mg.
„ „ of F_1	60 mg.

The frequency distribution of the F_2 generation will be seen in the following table.

TABLE XXVII.

Lint index (mg.)	Frequency
27	1
32	6
37	20
42	40
47	39
52	52
57	17
62	7
67	2
TOTAL	184

[1] Harland, S. C. (1925) *The Empire Cotton Growing Review*, Vol. II.
[2] Harland, S. C. (1920) *West India Bull.*, Vol. XVII.
[3] Kearney, T. H. loc. cit.

The distribution of lint index in the F_2 generation is worthy of comment. In the first place the frequency curve shown in Fig. 14 is unimodal and does not suggest

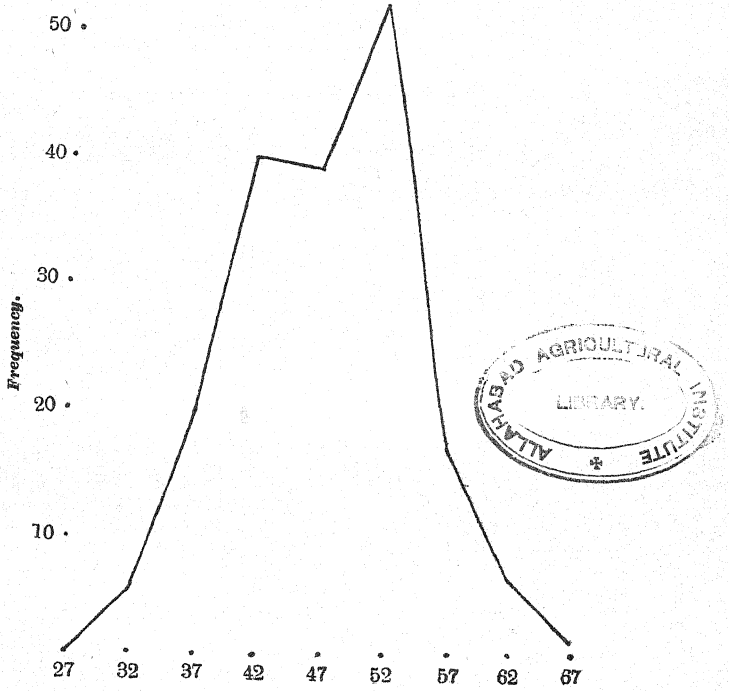


FIG. 14.

any factorial analysis. Then there is the decided inclination to produce plants with smaller lint index, but both the parent types have been recovered.

The figures of the F_3 generation are presented in the following Table :—

TABLE XXVIII.

Family No. (F_3)	Parental value (F_2)	Frequency arrays of lint index of F_3 families									Mean
		2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5	
2-6	2·9	2	..	3	15	11	9	4	2	..	4·4
2-2	3·7	..	..	3	3	2	..	..	..	..	3·9
2-5	3·7	..	..	1	4	..	..	..	..	..	3·9
2-1	3·8	..	..	1	3	1	..	..	..	..	4·0
1-6	4·0	..	1	5	6	1	2	..	..	..	4·0
1-7	4·1	..	..	..	3	3	3	1	..	..	4·6
1-9	4·3	1	2	2	3	1	2	..	1	..	4·0
1-1	4·8	..	..	5	15	12	..	..	..	..	4·1
1-2	4·0	..	..	..	3	5	4	1	1	..	4·7
1-8	5·0	..	1	1	6	..	1	3	1	1	4·6
1-3	5·1	..	..	..	1	..	..	1	..	..	4·7
1-4	5·2	..	..	2	8	12	12	4	2	..	4·7
2-4	5·3	..	..	..	1	..	4	1	1	..	5·1

An examination of the above table will reveal that the splitting here is very comparable to that of seed index. All the families, with the possible exception of 1-1, show splitting in a very indefinite way. The presence of *plus* and *minus* modifying genes is also clear. Seven of the thirteen families have means lower, one the same and the rest five higher than the parental value.

CORRELATIONS.

A very large number of correlations of the lint and seed characters with other more easily recognizable morphological characters have been pointed out by many workers. These correlations, apart from their scientific value, are of great service in commercial cotton breeding. The following correlations were worked out in the present case :—

1. The correlation coefficient of lint-length and lint index was—0·024 $\pm$ 0·051. These figures are not at all significant.
2. The coefficient of correlation of lint-length and seed index was +0·163 $\pm$ 0·048. These figures are also not significant.
3. The coefficient of correlation of lint-length and boll-length was —0·067 $\pm$0·051. It has been frequently asserted that plants with long bolls produce long lint. This statement is not amplified in the present case as long lint occurred both on plants with long and short bolls.
4. Length of lint and petal length. The coefficient of correlation between these two characters was +0·521 $\pm$0·048.

5. The coefficient of correlation between length of lint and length of leaf was $+0{\cdot}125 \pm 0{\cdot}201$.
6. The seed index was found to be very highly correlated with lint index; the coefficient of correlation was $+0{\cdot}710 \pm 0{\cdot}024$.
7. The coefficient of correlation between seed index and length of boll was $+0{\cdot}428 \pm 0{\cdot}053$.

Summary.

The mode of inheritance of nine characters was worked out in a cross between *G. cernuum* and *G. indicum* (Burma Silky).

G. cernuum had shallow broad-lobed leaves, long bracts, petals and bolls, very short lint and very high lint index and seed weight.

Burma Silky had shallow broad-lobed leaves, short bracts, petals and bolls, long lint and low lint index and seed weight.

The F_1 did not show heterosis except in the case of length of petiole. In the case of leaf factor, leaf-lobe index, index of lowest sinus-breadth, length of bracts, length of petals, length of bolls and seed index, the F_1 was intermediate, while a tendency to dominance of Burma Silky parent was shown in the case of width of bolls and length of lint and of the *Cernuum* parent in the case of lint index.

The depth of laciniation was inherited in a simple manner. The length and breadth of bracts showed very little splitting in the F_3, while the rest of the characters were very much complicated.

From the correlations worked out, it was noticed that the length of lint was inherited independently of lint index and seed index and that lint index was very highly correlated with seed index. It would, therefore, seem quite possible to combine these three highly desirable characters.

Acknowledgments.

I am highly indebted to Professor E. E. Cheesman under whose supervision the work was carried out. His willingness to help and his genuine interest in the work did more than anything else to the better understanding of the several problems.

To Dr. Harland I am indebted for very helpful suggestions and criticisms during the progress of the work. I am also thankful for his suggestions in writing up this paper.

Mr. J. B. Hutchinson provided the seeds of the two parent types and the F_1 generation, for which I am thankful to him.

I am also obliged to the Rev. Kenderick R. Bhagan for useful criticism during the preparation of the paper.

MGIPC—M—III-1-84—4-2-30—600.

COTTON GROWING IN INDIA IN RELATION TO CLIMATE.

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(Received for publication on the 10th February 1930.)

INTRODUCTORY.

The ready response of the cotton plant to its environmental factors has long been realized. Of these, the climatic factor is the most important as it is least amenable to correction by human agency. Literature on cotton is full of allusions to the effects of climate on its growth and maturation. It is also recognized now that climatic conditions are the chief determinants of the variety of cotton which will grow successfully in any locality. The study of the "environmental parallels" will thus provide, perhaps, the safest key to the introduction of exotic types.

So far concerted efforts for a proper climatological survey of the globe have not been made and it is the object of this paper to present, in a condensed form, the climatic conditions under which cotton is grown in India[1]. It is, however, recognized that more meteorological data are required before the present survey can be considered comprehensive.

The places dealt with have been deliberately chosen and only represent some of the different cotton growing regions in India. The Central Provinces—one of the most important areas—are omitted. Indore is a typical black cotton soil area, however, with somewhat similar meteorological conditions to parts of the Central Provinces, and may serve instead as an example. (See Cotton map of India; Report of the Indian Cotton Committee, 1919.) The localities chosen are:—

Lyallpur	Punjab Canal Colonies.
Hyderabad . . .	Sind.
Cawnpore	Cotton Growing areas in the United Provinces.
Surat	Broach Deshi Tract.
Indore	Central Indian States.
Belgaum	Deccan Tract (Bombay Presidency).
Coimbatore. . . .	Deccan Tract (Madras Presidency).

[1] Walker, G. T. (1914). *Mem. Ind. Meteo. Dept.*, Vol. XXII, Part III.

It will be seen from the various diagrams herein presented that the time of sowing of cotton at various places has been so adjusted as to provide the best possible climatic environment to the crop. A fair amount of rain (in non-irrigated areas) and high minimum temperatures during the growing period are essential to its proper development, while sunny and dry conditions are favourable for the picking season.

Williams[1],[2] in Egypt was the first to draw attention to this phase of the subject. His diagrams of the climatic conditions of the various cotton growing countries of the world are important.

Canney [3] has made a general survey of the world with regard to temperature and the amount of cloud and has indicated that "the acreage in more suitable climates is probably at least as large again as the total present acreage under rainfall cotton."

In another paper, Canney [4] has considered the question of climatic severity in relation to the varieties suited to particular zones, paying particular attention to staple quality and length. He comes to the conclusion that staple quality is associated with the severity of climate and it is only asking for failure and disappointment to try and introduce a variety with a particular staple into an area whose climate is unsuited to that staple. He points out the relationships between growing period, length of staple, and climate and emphasizes the importance of a study of the natural environment of a type, when the question of the introduction of that type into another area is under consideration.

This paper of Canney deserves the careful consideration of all plant breeders, though more climatological data would be required before all Canney's suggestions could be accepted. His suggestion, for example, about the suitability of Sind for an Upper Egyptian type of cotton, if the sowing date were delayed, may be correct, but for a reason different from the one he puts forward. There are no early rains in the cotton growing areas in Sind, but other reasons, which also apply to some extent to the Punjab, may make it desirable to delay sowing, both in Sind and the Punjab. A detailed account of these reasons so far as the Punjab is concerned is in preparation in connection with sowing date experiments at Lyallpur.*

Trought [5] in his paper on the "Effects of some meteorological conditions on the growth of Punjab-American cotton" has drawn the mean temperature diagram of the Punjab and compared it with Williams' diagram of various countries. This diagram shows that the environment during the early stages of development

* This paper has now been written and the experimental data show that cottons sown in mid-June grow faster, flower more freely and open more bolls than earlier or later sown cottons.

[1] Williams, C. B. (1923). *Ministry Agri., Egypt, Tech. and Sci. Service, Bull.* No. 32.

[2] Williams, C. B. (1924). *Ministry Agri., Egypt, Tech. and Sci. Service, Bull.* No. 47.

[3] Canney, E. E. (1924). *Shirley Inst. Mem.*, Vol. 3.

[4] Canney, E. E. (1927). *Jour. Tex. Inst.*, Vol. XVIII.

[5] Trought, T. Effects of some meteorological conditions on the growth of Punjab-American Cotton. *Mem. Dept. Agri. Bot. Ser.* Vol. XVII, No. 6. (*In the press*).

of the crop when sown at what is considered to be the usual sowing date, namely, early May, is more exacting than in probably any other cotton growing country, at the same stage. This means that root development, as well as shoot growth, is detrimentally affected, with the possibilities of consequent harmful reactions in the crop's later development.

The advantages which are gained by a later sowing of cotton are now attracting notice, and figures have already been published by Roberts [1] showing the increased yields obtained on a field scale at Khanewal with later sowings. The avoidance of these extreme conditions can at least partially explain the advantages which in practice on quite a large scale have been shown to exist.

Presentation of results.

The method followed in plotting the different diagrams is precisely the same as that employed by Williams. This method is very convenient and has the advantage of comparison with Williams' diagrams of the different countries.

Complete data for the following factors are available and only these are dealt with—

1. Normal mean monthly maximum temperature.
2. Normal mean monthly minimum temperature.
3. Normal monthly rainfall.
4. Normal number of rainy days per month.
5. Normal monthly amount of cloud.
6. Normal mean relative humidity at 8 A.M.
7. Normal mean velocity of wind in miles per hour.

[1] Roberts, W. (1929). *Agri. Jour. India*, Vol. XXIV, Part II.

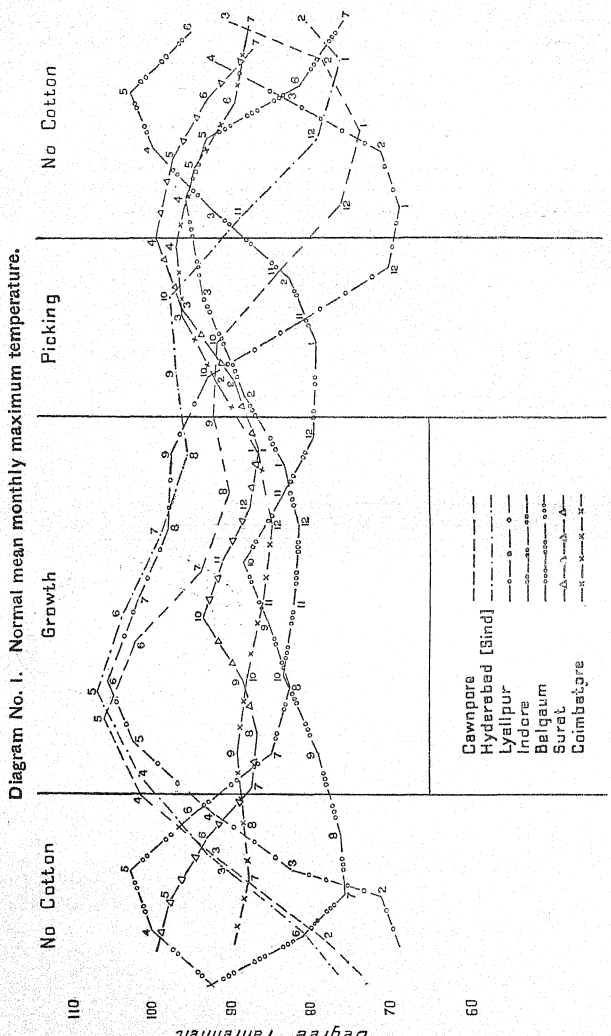


Diagram No. I. Normal mean monthly maximum temperature.
No Cotton
Growth
Picking
No Cotton
Degree Fahrenheit
110
100
90
80
70
60
Cawnpore
Hyderabad [Sind]
Lyallpur
Indore
Belgaum
Surat
Coimbatore

1. Normal mean monthly maximum temperature.

(Diagram No. 1.)

At Cawnpore, Hyderabad (Sind) and Lyallpur cotton is a summer crop, and is planted during the period of rising temperatures. The maximum temperature falls off during the later half of the growing season. During the picking season the maximum temperature falls off very quickly at both Cawnpore and Lyallpur and more slowly at Hyderabad. It is interesting to note that Lyallpur which starts with a very high temperature just after planting time has the lowest maximum temperature at the close of the season. It may also be remarked that the maximum temperature at Coimbatore does not vary more than 5 degrees during the whole of the growing season. The curve for Belgaum is comparable to that for Coimbatore. The curves for Surat and Indore are quite distinct from the others. The maximum temperature rises during the first half of the growing season, falls during the later half, and rises again during picking time. The spell of hot weather in the middle of the growing season is probably of little importance to the cotton grower. These curves resemble those for the mean temperature at Wad Medani in the Sudan as plotted by Williams. The similarity of conditions as regards maximum temperature is greatest just before picking commences.

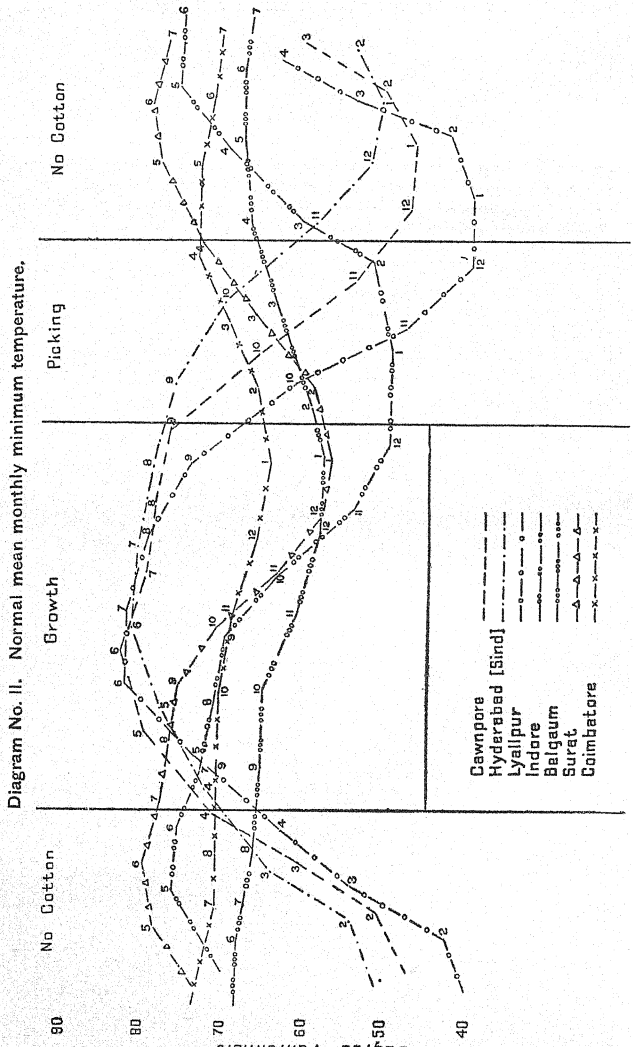


Diagram No. II. Normal mean monthly minimum temperature.
No Cotton
Growth
Picking
No Cotton
Degree Fahrenheit
90
80
70
60
50
40
Cawnpore
Hyderabad [Sind]
Lyallpur
Indore
Belgaum
Surat
Coimbatore

2. Normal mean monthly minimum temperature.

(Diagram No. 2.)

The normal mean monthly minimum temperature curves resemble those of the normal maximum temperature. Both at the planting time and at the close of the season, Surat has the highest minimum temperature, while Lyallpur has the lowest at the corresponding times. Like the maximum temperature, Coimbatore maintains a fairly constant minimum temperature during the growing season and a rising minimum temperature during the picking season. The spell of hot weather during the middle of the growing season at Surat and Indore does not apparently affect the minimum temperatures.

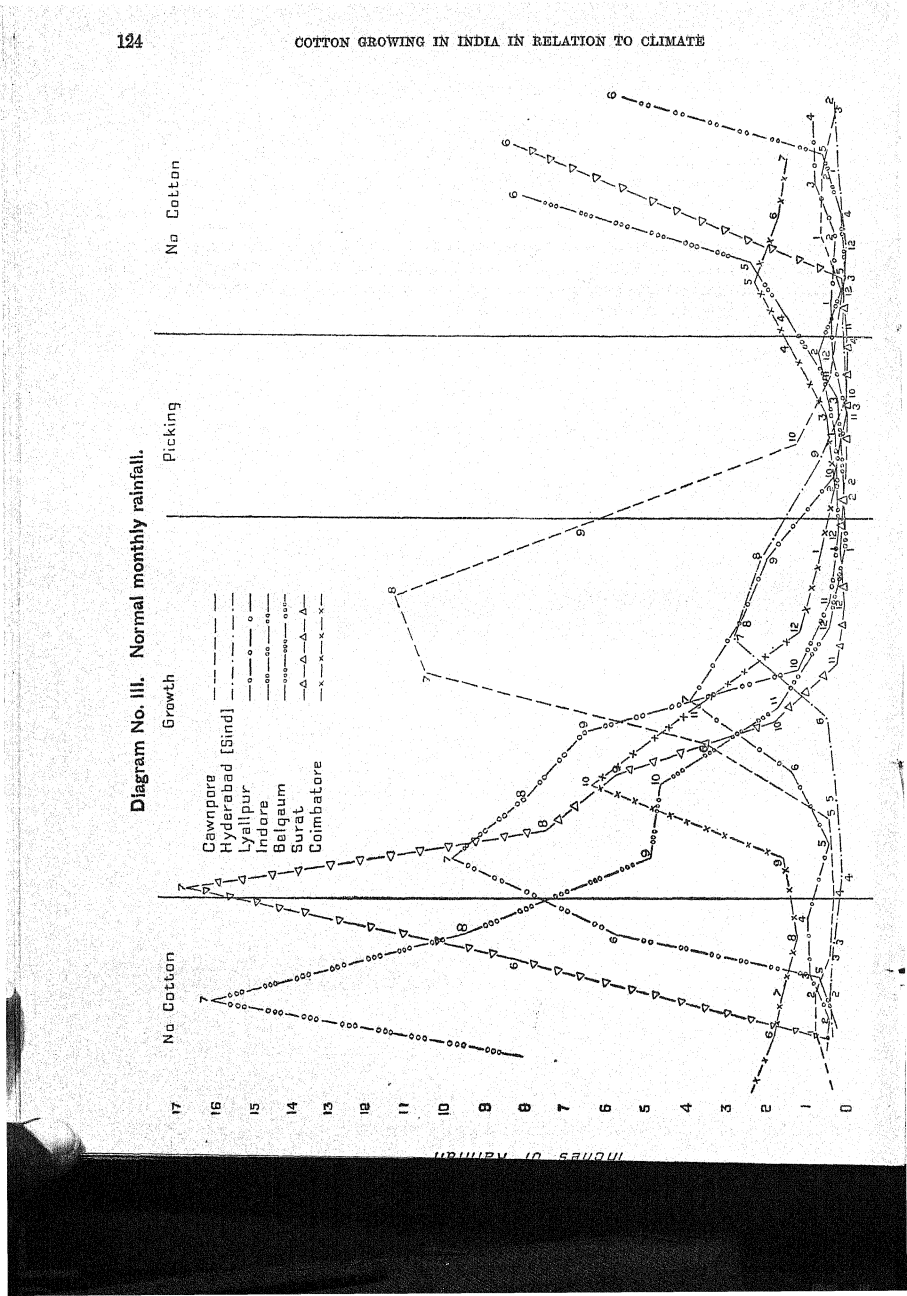


Diagram No. III. Normal monthly rainfall.
No Cotton
Growth
Picking
No Cotton
Cawnpore
Hyderabad [Sind]
Lyallpur
Indore
Belgaum
Surat
Coimbatore
Inches of Rainfall.
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

3. Normal monthly rainfall.

(Diagram No. 3.)

The time of planting of cotton is so adjusted as to get the maximum benefit of rainfall in the growing season and at the same time leave the picking season dry. The subjoined table will illustrate this point.

Table.

Name of place	Total annual normal rainfall (inches)	Rainfall during growing season (inches)	Rainfall during picking season (inches)	Rainfall during off season (inches)	Remarks
Belgaum . . .	50·13	9·37	1·50	39·26	No irrigation.
Surat	40·88	17·50	0·10	23·28	Do.
Cawnpore . . .	36·06	32·39	1·76	1·91	Do.
Indore	33·68	12·39	0·94	20·35	Do.
Coimbatore . . .	22·19	12·90	2·34	6·95	Irrigation.
Lyallpur . . .	13·38	10·40	0·72	2·26	Do.
Hyderabad . . .	7·82	5·92	0·38	0·82	Do.

At places where cotton is a rain-fed crop most of the rain which falls during the off-season is conserved in the soil and is used up by the plants. It will also be clear from the above table that at most of the places very little rain falls during the picking season. This is conducive to easy and clean picking. At places like Lyallpur, Hyderabad and Coimbatore where rainfall is insufficient for the requirements of the plants, artificial irrigations are given to supply the proper amount of moisture to the growing plants. Figures of the actual amount of water applied in irrigations are not available except for Lyallpur where the amount varies from 10 to 20 acre-inches in the season.

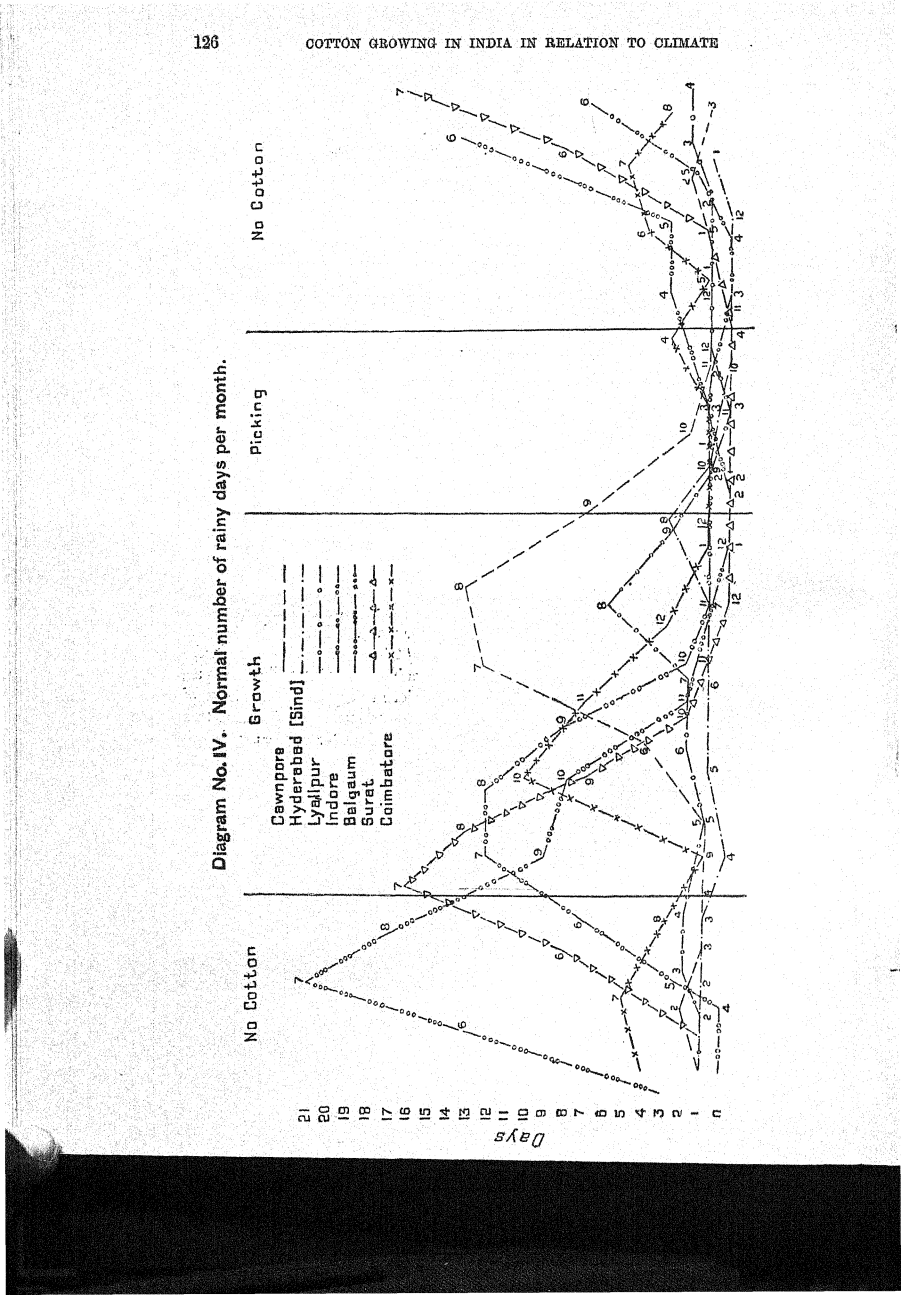


Diagram No. IV. Normal number of rainy days per month.
No Cotton
Growth
Picking
No Cotton
Cawnpore
Hyderabad [Sind]
Lyallpur
Indore
Belgaum
Surat
Coimbatore
Days
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

4. Normal number of rainy days per month.

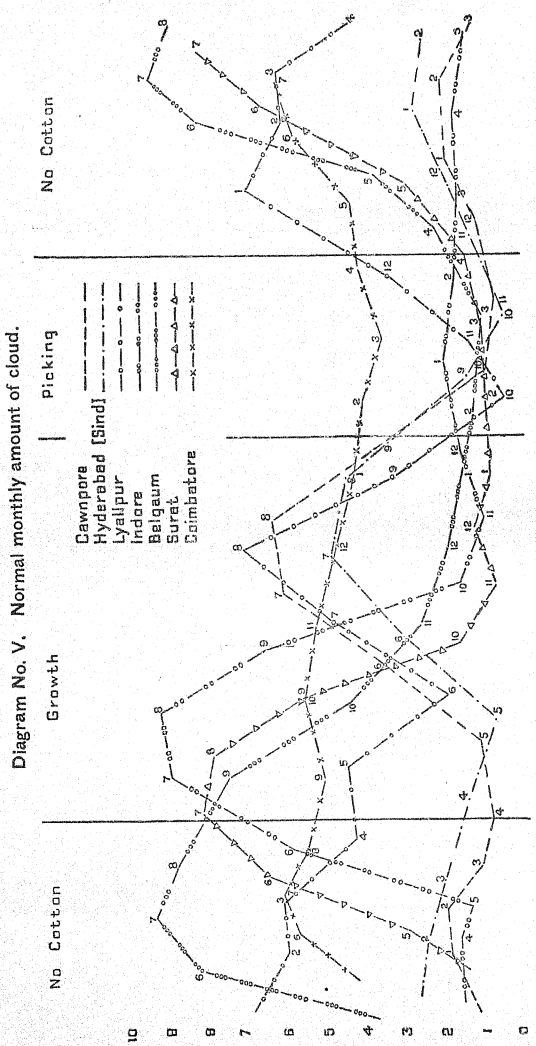
(Diagram No. 4.)

The utility of rain to the crops is determined by its amount and distribution and its retention in the soil at a depth where it is available for the plant. Here we are only concerned with the first phase of the question as the retention of the rain water in the soil is a matter of soil type and of agricultural practice and in the areas where the ryot depends entirely on rain he usually takes good care not to allow any unnecessary waste of water.

Excessive showers within a short period are far less useful than lighter showers well distributed; the curves of the normal number of rainy days are thus of interest.

It would appear that, among the rain-fed areas, sowing may be delayed at Cawnpore for lack of proper rainfall, but once the crop is established it is not likely to suffer from lack of moisture. The other features of this curve resemble those of the rainfall curve. The number of rainy days at different places is in direct proportion to the amount of rain.

Diagram No. V. Normal monthly amount of cloud.

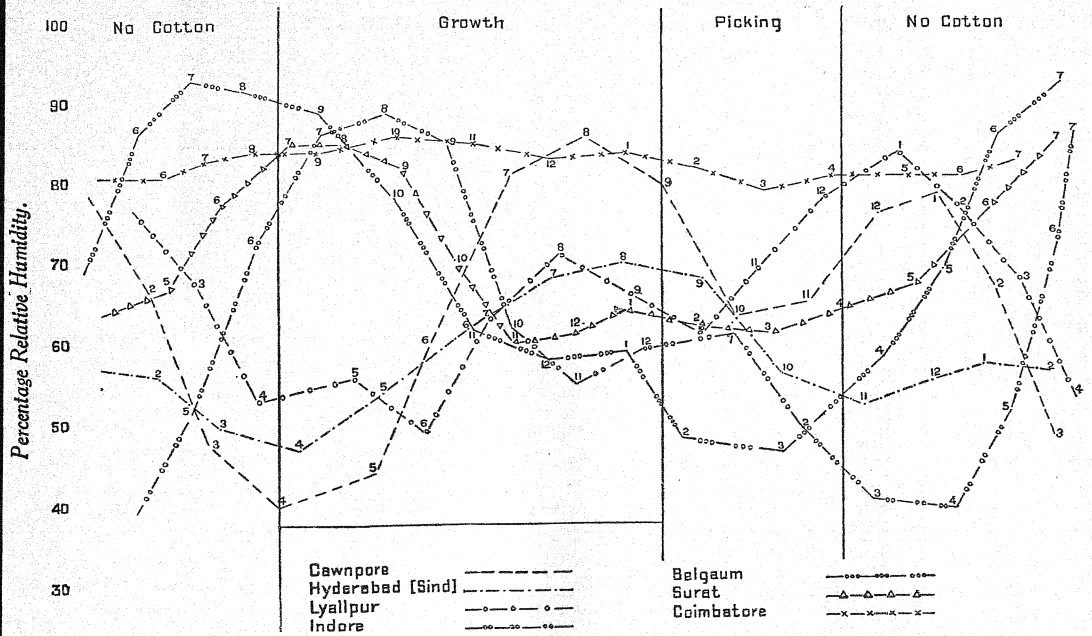


5. Normal monthly amount of cloud.

(Diagram No. 5.)

The amount of rainfall and the number of rainy days are a function of the amount of cloud. Clouds also tend to reduce air and soil temperatures, the intensity of light, and the amount of evaporation. At sowing time there is sunny weather at Cawnpore and Hyderabad, moderately cloudy at Lyallpur and Coimbatore and heavily overcast at Indore, Surat and Belgaum. Towards picking time the amount of cloud decreases appreciably at all places except at Coimbatore, thus enabling the cotton to be picked during dry sunny weather. The amount of cloud again increases at all places towards the close of picking season.

Diagram No. VI. Normal mean relative humidity.



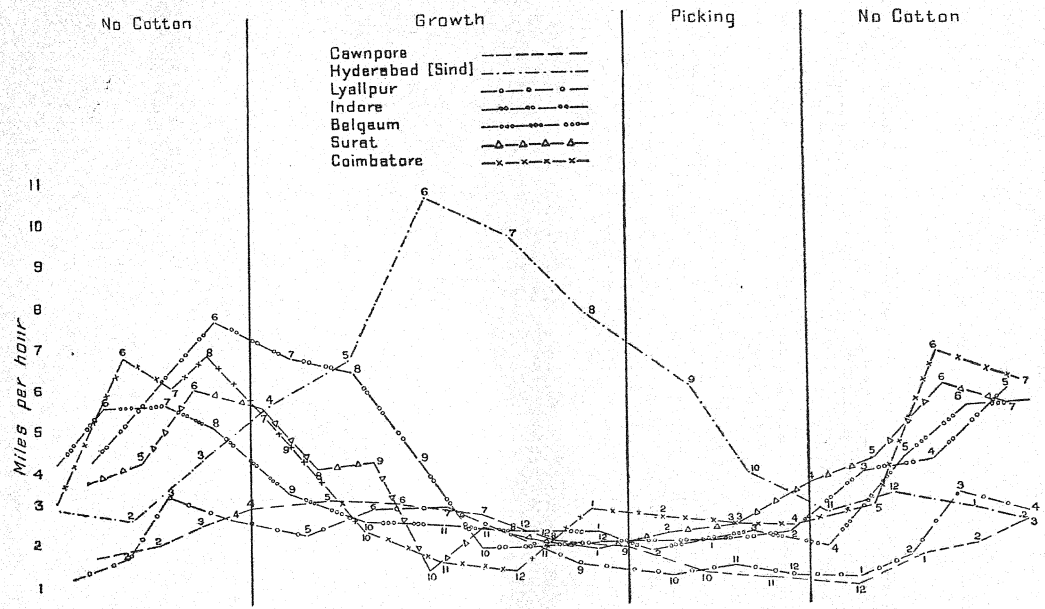
6. Normal mean relative humidity.

(Diagram No. 6.)

Relative humidity is very high at Surat, Indore and Belgaum and fairly low at Lyallpur, Cawnpore and Hyderabad at the start of the growing season. At the former three places it decreases steadily with the increase in maximum temperature. At Cawnpore and Hyderabad it increases during the growing season, while the maximum temperature decreases. At Lyallpur conditions are somewhat similar to those obtained at Cawnpore and Hyderabad but the effect is not so marked. Coimbatore maintains a relative humidity above 80 per cent. and below 86 per cent. throughout the season. One point of interest is that the relative humidity at Indore and Belgaum—the places of highest relative humidity at the start—falls to a very low value at the end of the picking season.

The figures presented in Diagram No. 6 are those of the relative humidity at 8 A.M. and are of doubtful value as the relative humidity is constantly changing throughout the course of the day. For this reason the relative humidity at Lyallpur is apparently very high during November and December. This is solely due to the effect of dew and haze in the mornings during these months. After the dew evaporates which rarely takes place before 8 A.M., the atmosphere gets very dry indeed.

Diagram No. VII. Mean velocity of wind in miles per hour.



7. Mean velocity of wind in miles per hour.

(Diagram No. 7.)

During the first half of the growing season the mean wind velocity is fairly high at all places except Lyallpur and Cawnpore. The wind velocity at Hyderabad is conspicuously high and remains so throughout the growing and picking season. At the rest of the places the wind velocity is always less than 3 miles per hour during the later half of the growing season and throughout the picking season.

Summary.

An attempt has been made to compare the climatic conditions prevailing in typical cotton growing localities in India. The different factors have been treated separately, but it is recognized that the effect of each depends on the other. Information of this sort will, it is hoped, contribute to a proper understanding of the various ecological problems of the cotton plant and its pests.

The greatest similarity of conditions at the various places is during the middle of the picking season, and not about a month or so before picking as found by Williams in Egypt and the Sudan.

Rainfall is low in the later half of the growing and throughout the picking seasons.

Realtive humidity varies enormously at different places during the course of the year.

Acknowledgments.

The writers' thanks are due to the several officers of the Provincial Departments of Agriculture who supplied information regarding the time of sowing and picking of cotton and other information about the crop in their localities.

This work was carried out as a part of the Indian Central Cotton Committee's Research Scheme (Botanical) in conjunction with the Punjab Government.

LOCALITIES.

—	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
1. LYALLPUR.												
Normal maximum temperature in °F	68·8	71·2	82·6	92·1	102·6	105·9	102·3	98·3	97·6	93·0	81·3	70·1
,, minimum temperature in °F	39·5	42·2	53·5	62·6	75·5	82·2	82·3	79·6	74·5	61·2	47·6	39·8
,, rainfall in inches	0·38	0·31	0·80	0·88	0·38	1·33	3·85	2·75	2·0	0·34	0·01	0·35
,, number of rainy days	1	1	2	2	1	3	2	6	3	1	0	1
,, cloud	6·0	6·0	6·1	4·3	4·5	2·0	4·3	7·1	3·4	0·5	1·4	3·4
,, relative humidity (per cent)	84	77	68	53	56	49	63	71	66	61	69	78
,, wind velocity in miles per hour.	1·1	1·6	3·1	2·6	2·2	2·8	2·9	2·2	1·5	1·2	1·4	1·2
2. CAWNPORE.												
Normal maximum temperature in °F	73·5	78·3	90·2	101·5	106·2	102·6	93·7	90·4	92·3	91·8	84·5	76·0
,, minimum temperature in °F	46·7	50·6	60·2	71·4	79·6	82·9	80·1	78·6	76·7	65·9	54·4	47·2
,, rainfall in inches	0·65	0·66	0·30	0·21	0·4	3·32	10·55	11·38	6·74	1·27	0·40	0·18
,, number of rainy days	1	2	1	1	1	4	12	13	7	2	1	1
,, cloud	1·9	2·0	1·2	0·9	1·2	3·5	6·1	6·4	3·6	1·0	0·7	1·2
,, relative humidity (per cent)	79	67	48	40	44	60	81	86	80	63	65	76
,, wind velocity in miles per hour.	1·6	1·9	2·4	2·8	3·0	2·9	2·7	2·0	1·9	1·2	1·1	0·9
3. HYDERABAD (SIND).												
Normal maximum temperature in °F	76·2	80·5	92·6	102·0	107·2	104·2	99·2	95·9	97·4	97·9	89·1	79·6
,, minimum temperature in °F	50·6	54·0	64·0	72·1	78·2	81·7	80·9	79·0	76·2	70·1	58·9	52·3
,, rainfall in inches	0·20	0·27	0·24	0·05	0·20	0·45	2·85	2·12	0·60	0·02	0·06	0·06
,, number of rainy days	1	1	1	0	1	1	1	3	1	0	0	0
,, cloud	2·7	2·5	2·1	1·5	0·8	3·0	4·9	4·5	1·5	0·5	1·3	2·0
,, relative humidity (per cent)	57	56	50	47	54	62	68	70	68	56	52	55
,, wind velocity in miles per hour.	2·8	2·5	4·0	5·4	6·5	10·6	9·7	7·7	5·9	3·7	2·7	3·1

4. Surat.

Normal maximum temperature in °F	86·6	89·6	90·3	90·8	87·7	83·4	87·4	86·7	88·6	93·7	91·4	87·7
,, minimum temperature in °F	56·9	59·1	66·2	73·1	78·4	79·6	77·6	76·7	75·7	71·2	63·5	58·3
,, rainfall in inches	0·14	0·07	0·02	0·04	0·24	8·34	16·70	7·55	5·80	1·75	0·19	0·04
,, number of rainy days	0	0	0	0	1	8	16	13	7	2	1	0
,, cloud	0·9	1·0	1·1	1·5	2·9	6·5	8·1	7·9	5·7	1·7	0·8	1·3
,, relative humidity (per cent)	64	62	61	64	67	77	85	85	82	70	60	61
,, wind velocity in miles per hour	1·9	2·2	2·4	3·4	4·0	5·8	5·3	3·8	4·0	1·3	2·4	2·0

5. Belgaum.

Normal maximum temperature in °F	83·5	88·3	93·7	95·9	93·1	81·2	75·7	76·3	79·0	83·2	82·4	81·7
,, minimum temperature in °F	57·6	59·4	63·9	67·1	68·1	68·0	67·0	66·3	65·3	65·1	61·1	58·3
,, rainfall in inches	0·13	0·05	0·27	1·60	2·46	8·14	16·15	9·67	4·88	4·67	1·74	0·37
,, number of rainy days	0	0	1	3	3	13	21	17	9	8	2	1
,, cloud	1·6	1·3	1·1	2·2	3·7	8·1	9·3	8·8	7·5	4·5	2·7	2·0
,, relative humidity (per cent)	59	48	46	58	69	86	93	92	89	78	62	58
,, wind velocity in miles per hour	2·2	1·8	2·2	2·0	3·9	5·3	5·4	4·9	3·2	2·5	2·4	2·3

6. Indore.

Normal maximum temperature in °F	79·4	82·8	92·3	100·0	102·9	95·3	85·1	82·6	85·1	88·5	83·7	79·7
,, minimum temperature in °F	49·6	51·9	60·4	69·8	76·2	75·6	72·7	71·4	70·0	63·3	54·3	49·6
,, rainfall in inches	0·28	0·72	0·08	0·14	0·58	5·70	9·87	8·01	6·53	1·15	0·41	0·21
,, number of rainy days	1	1	0	0	2	7	12	12	8	2	1	1
,, cloud	2·0	1·7	1·6	1·7	1·4	5·9	8·9	9·2	6·6	1·7	1·1	1·6
,, relative humidity (per cent)	61	49	40	39	51	72	86	89	85	62	55	59
,, wind velocity in miles per hour	2·6	2·2	3·6	4·0	5·7	7·5	6·6	6·2	4·1	1·9	1·9	2·0

LOCALITIES—*contd.*

	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
7. COIMBATORE.												
Normal maximum temperature in °F	88·7	91·7	90·2	97·2	94·8	89·6	87·7	88·2	89·1	88·1	86·0	85·0
,, minimum temperature in °F	64·4	65·9	69·8	73·5	73·5	71·8	70·8	70·8	70·7	70·6	68·8	65·8
,, rainfall in inches	0·59	0·32	0·48	1·44	2·26	1·66	1·46	1·13	1·51	6·41	3·75	1·18
,, number of rainy days	1	1	1	3	1	4	5	3	1	10	7	3
,, cloud	4·3	4·1	3·6	4·1	4·3	5·7	6·1	5·5	5·1	5·6	5·3	4·8
,, relative humidity (per cent)	84	82	79	81	81	81	83	84	84	86	85	83
,, wind velocity	2·8	2·6	2·4	2·4	2·0	6·6	5·9	6·7	4·5	2·4	1·5	1·3

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THE EFFECT OF SOME METEOROLOGICAL CONDITIONS ON THE GROWTH OF PUNJAB-AMERICAN COTTON.*

BY

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(Received for publication on 7th March 1930.)

INTRODUCTION.

In this paper after reviewing generally the Punjab climate in the cotton season I propose to discuss briefly the following points :—the particular effect on the daily increase in height of Punjab-American cotton of (1) irrigation and rainfall, (2) evaporation, (3) duststorms, (4) soil temperatures, and (5) maximum and minimum air temperatures.

GENERAL REVIEW OF PUNJAB CLIMATE.

The cotton season in the Punjab is from April to December or January. Sowing takes place in April and May and the last pickings sometimes are not harvested until towards the end of January.

In general, it is beginning to become warm in April; May and June are hot and dry: July and August are hot and damp, September and October are still warm during the day but minimum temperatures are dropping rapidly. In November, December and January, which are typically winter months, the atmosphere is dry.

In December and January particularly the days are clear and cold. During December there is a general expectation of showers of rain.

Table I shows the monthly normals of maximum and minimum temperature for four places in the Punjab, namely, Sirsa, Multan, Montgomery and Lahore, compared with other cotton growing localities in different parts of the world. Mr. C. B. Williams (10)† has given diagrams for different places showing the relation of the mean maximum temperatures to the periods during which the cotton is growing, the start of picking and the harvesting. If the Lahore mean temperatures are inserted on these diagrams, it is seen that the Punjab is distinctly abnormal, though the temperatures at first picking time approach the temperatures at the start of picking for the other countries as recorded by Mr. Williams.

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* Revised from a paper written for the Indian Science Congress held at Madras, January 1929.

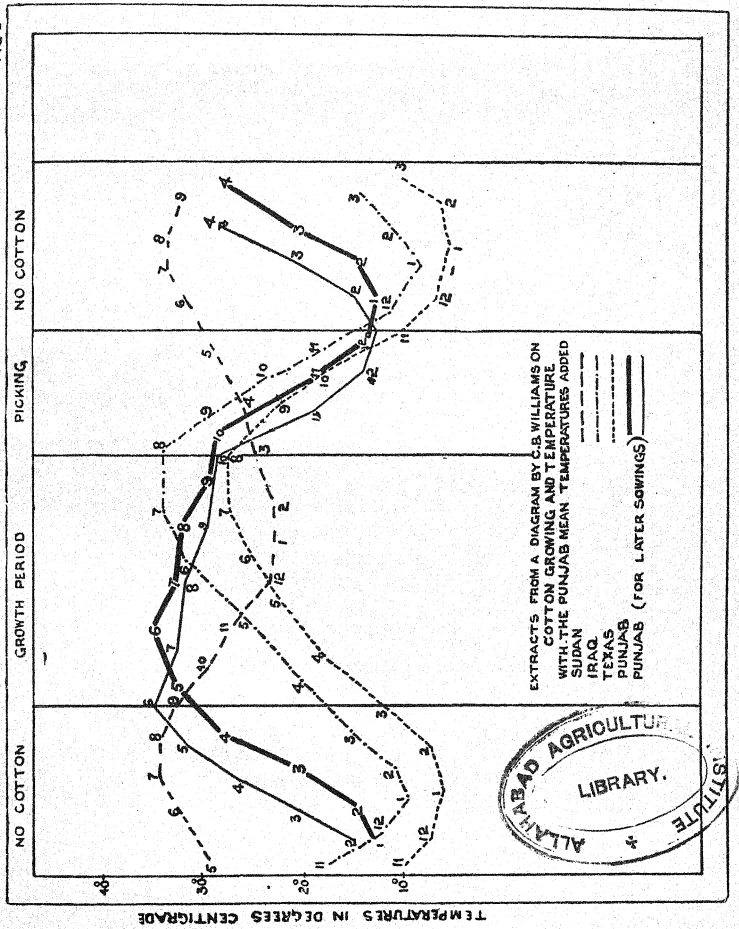
† Reference is made by number to Literature cited, p. 153.

TABLE I.

Mean monthly maximum and minimum temperature in degrees Fahrenheit.

Place	January	February	March	April	May	June	July	August	September	October	November	December
	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.
Chitaldrug . .	82.7 61.8	87.5 63.5	92.0 69.3	97.6 72.5	95.5 70.1	89·1 70.3	78.3 68.1	80.9 68.0	83.5 67.9	88.2 68.6	87.7 65.0	84.9 62.8
Charleston . . .	56.6 41.2	57·2 41·8	61.4 46.6	71.4 56.7	80.2 64.6	88.2 74.1	87.8 74.7	88.9 75.4	80.2 68.9	72.5 59.0	67.1 50.4	64.2 49.6
Mobile	57.5 37.4	60.6 44·3	64.2 47.7	74.1 59.1	78.7 63.4	89.5 73.5	90.9 73.1	93.0 74.6	84.1 68.5	78.7 58.2	71.6 51.6	64·5 51.9
Sacaton . .	64.7 30.6	73.8 36.6	70.1 39.5	80.0 45.9	94.8 57.9	103.8 65.5	102.0 73.6	103.6 71.8	99.0 65.5	87.0 48.1	78.6 38.7	63.0 34.2
Gatooma .	95.7 59.9	87.3 61.3	87.4 63.6	82.1 54.6	86.8 52.6	75·1 48.4	78.7 41.7	83.6 46.9	90·2 56.7	95.8 59.6	97.4 64.9	90.6 61.7
Sirsa . .	70.1 42.7	74.5 46.6	87.3 56.9	99.4 67.8	106.8 77.4	106.9 83.6	100.5 81.9	98.1 80.5	98.1 75.9	96.0 63.2	85.1 50.3	74.2 43.0
Lahore . .	68.4 41.1	72.1 44.5	83.9 54.5	96.5 64.5	105.4 73.4	107.4 80.1	100.5 80.2	97.8 78.9	98.3 73.4	94.9 60.3	83.9 48.2	72.8 40.9
Montgomery . .	68.4 41.3	72.5 45.4	84.4 55.7	97.2 66.9	107.2 78.0	109·1 83.9	104·3 83.9	101.8 82.1	100.6 76.1	96.3 63.0	84.8 52.0	72.5 42.6
Multan . . .	69.7 43.3	73.6 47.3	85.8 58.2	97.9 68.4	106.9 78.1	108.3 84.2	104.3 84.3	101·2 82.6	100.5 77.5	95.9 65.1	84.8 53.5	73.5 45.0
Giza .	66.4 41.9	69.6 43.3	75.0 46.9	82.6 52.7	89.0 57.9	93.6 63.5	95.2 66.9	93.6 67.6	88.7 63.8	85.0 60.5	77.3 53.4	69·1 45.8
Khartoum .	85.6 58.4	88.8 60.8	95.5 64.7	102.2 70.8	106.7 77·2	107.2 78.6	103.3 77.2	99.8 76.6	102.6 77.0	102.0 75.0	94.4 68.5	86.7 60.6
Bagdad .	59.0 38.8	64.8 42.8	72.7 49.6	83.3 58.5	93.8 68.1	104·2 75.9	109.5 79.7	109.8 79.0	103.5 72.6	92.4 63 3	75.0 50.7	62.5 42.4

Fig. 1 shows a diagram drawn to show this comparison. During the early growth of the crop in the Punjab the young plants are subjected to higher temperature



conditions than anywhere else in the world, during their later growth the temperatures are still high, but at the beginning of the picking period the temperatures are somewhat similar to those in other localities. It may be noted, however, that, if the picking period were considered as being finished at the end of January instead of the end of December, the curve would show a greater similarity with curves in other localities. The picking period curve is thus to some extent arbitrary. It is interesting also to note that if there should be a change in the customary sowing date, that is, if cotton were sown in the middle of June or beginning of July, the temperature curve during the growth period, though still high, would be more in conformity with that of the Sudan. Cotton would be sown then on a falling temperature curve and would approximate more to a " Winter " crop as it is in the Sudan, than a " Summer " crop as it is at the present in the Punjab. There is a tendency in the Punjab to later sowings ; and observations made in 1927 in connection with the partial failure of the crop in that year showed almost invariably that cotton sown about the middle of June did better than earlier sown crops and showed few or no signs of failure.

This tendency to later sowings is explicable from Mr. Williams' diagrams. As the climate cannot be altered, the grower, as a result of experience, is altering the growth period to bring it more in conformity with those temperature conditions which experience has also found to be best for growing cotton in other countries. Experiments on sowing dates which are in progress may enable definite recommendations to be made on this point. In the years under consideration, the evaporation, from a free water surface, and as recorded by atmometers, decreases fairly steadily from the beginning of June, except in 1926, where the fall does not make itself apparent until mid-June. Young cotton plants are therefore not exposed to quite such stringent conditions during June as during May. This may partly account for the better results which were observed with later sown cottons in 1927.*

The incidence of rainfall during the cotton season is very variable. This is shown in Figure 2 which gives the rainfall for certain recent years. To get the full benefit of rain, the rain should be equally distributed throughout the growth season. An examination of the diagram shows that in the Punjab it is the exception rather than the rule for rain to be so distributed. Under irrigated conditions, however, it would be expected that a deficiency or irregularity of rainfall would be compensated for by irrigation. But the irrigation of cotton during " the Rains " is often neglected in the expectation that rain will come. Rain when it does come is also frequently very much localised. Differences of one or more inches often occur in the readings from rain gauges less than 5 miles apart.

* The same observations were also made in 1928, which was a " failure " year.

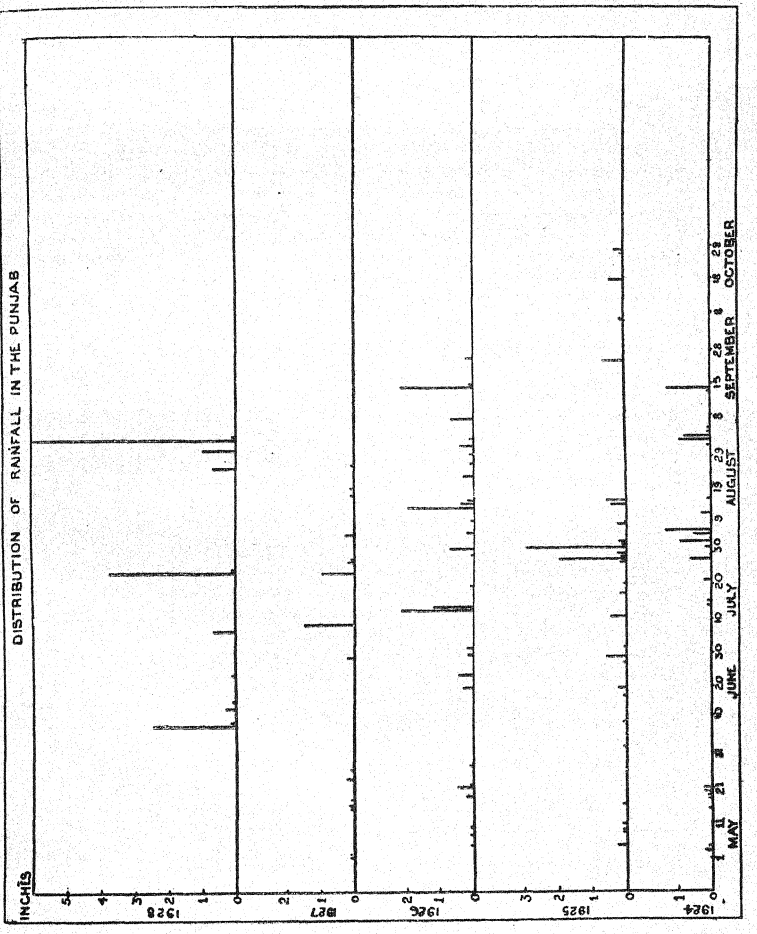

DISTRIBUTION OF RAINFALL IN THE PUNJAB
INCHES
1928
1827
1826
1825
1824
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

FIG. 11

The months of May and June are rendered unpleasant by frequent duststorms. Duststorms, also, are as a rule localised, and consist of winds which arise suddenly, frequently from a dead calm. They vary considerably in intensity and the air is heavily charged with dust. A severe duststorm occasionally affects a large area.

Frequently a little rain will follow a duststorm. Almost invariably a duststorm occurs in the evening. The earlier part of the day may have been either sunny throughout, or cloudy, but the approach of a duststorm is generally recognized by a characteristic oppressive feeling. Thunder and lightning are general features of duststorms. A lowering of temperature and a rise in relative humidity, even when no rain falls, almost invariably accompany a duststorm. At the time of a duststorm, the barograph shows an immediate but slight rise, irrespective of whether the barograph is falling or rising. (Observations in 1928 only).* The soil temperatures are unaffected when no rain accompanies the duststorm. Occasionally there is a slight reduction in the soil temperature at 5 cms. depth due, probably, to the increased evaporation from the soil on account of the wind.

We will now turn to the details of the data presented.

Increase in height curves of the variety 4F are presented in Figures 3, 4 and 5, for the years 1926, 1927, and 1928 respectively. The cotton was sown each year about the beginning of May. In general, it is found that the increase in height curve of 4F sown in early May and similar curves of other varieties sown at the same date (also 4F curves sown at different dates) agree so closely in their daily fluctuations though not necessarily in the amplitude of their fluctuations that I shall confine my attention, therefore, to the 4F curves from the crop sown in early May, as 4F provides very nearly the total area sown to Punjab-American Cotton in the Province and the beginning of May is a most usual sowing date.

Mr. Bailey and myself (2) consider that elongation is not necessarily an index of growth in mass, and though it seems that the two phenomena must be correlated, this limitation should be borne in mind. Our experiments supported the conclusions of other authors (McDougal, Livingston, Balls, etc.) that increase in length depended chiefly on the availablility of water to the plant. Recently published work in America by Veihmeyer and Hendrickson (9) also supports this suggestion. They say that increase in length depended entirely on the water used by the plant, and their figures give a correlation coefficient of $+ \cdot 955 \pm \cdot 002$ between "use of water" and growth in length.

Increase in height in the main stem of 4F, although 4F is very monopodial type, can be taken as an index of the general elongation of the plant. In Figure 6 it will be seen that the increase in length of monopodia conforms very nearly to the increase in length of the main stem.

* Confirmed in 1929.

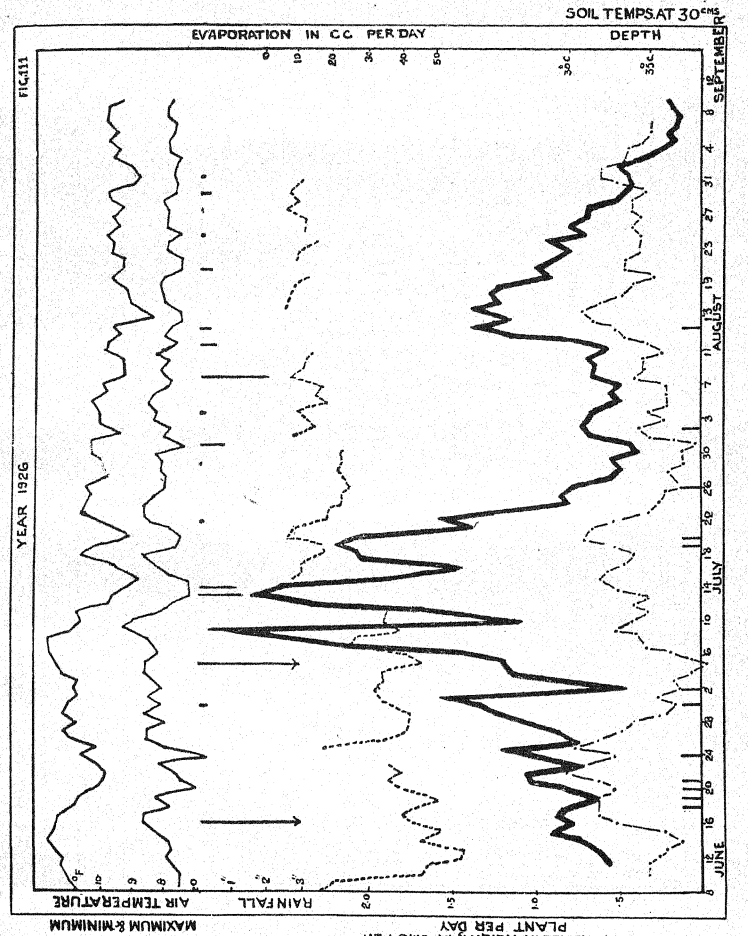


FIG.111
YEAR 1926
EVAPORATION IN C.C. PER DAY
SOIL TEMPS. AT 30 CMS DEPTH
RAIN FALL
AIR TEMPERATURE
MAXIMUM & MINIMUM
INCREASE IN HEIGHT IN CMS PER PLANT PER DAY
JUNE
JULY
AUGUST
SEPTEMBER

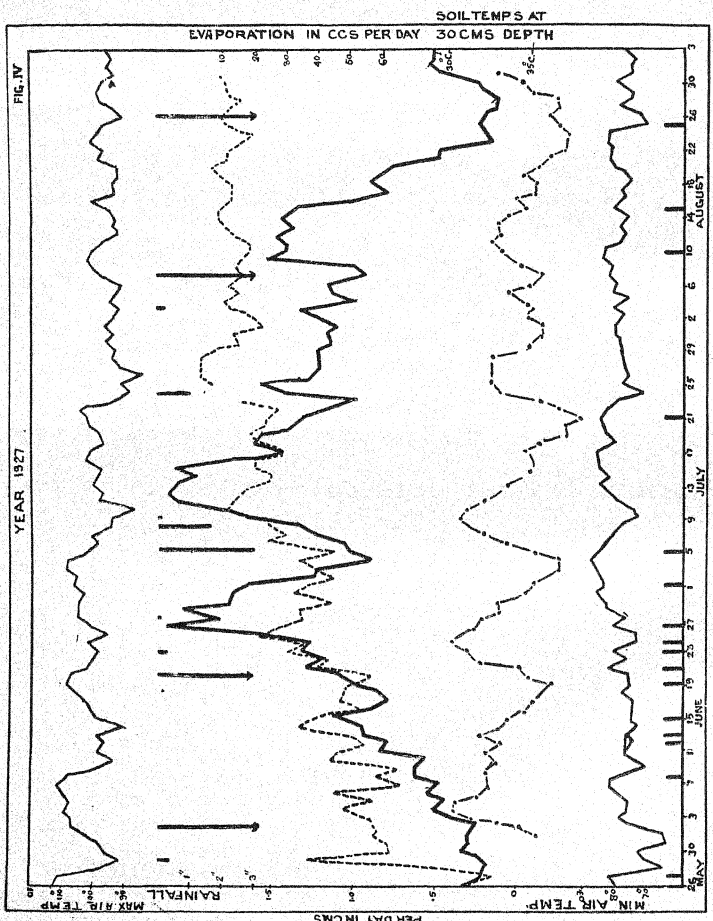


FIG. IV
YEAR 1927
SOIL TEMPS AT
EVAPORATION IN CCS PER DAY 30 CMS DEPTH
MAX. AIR TEMP.
RAINFALL
MIN. AIR TEMP.
MAY
JUNE
JULY
AUGUST
INCREASE IN HEIGHT PER PLANT
PER DAY IN CMS

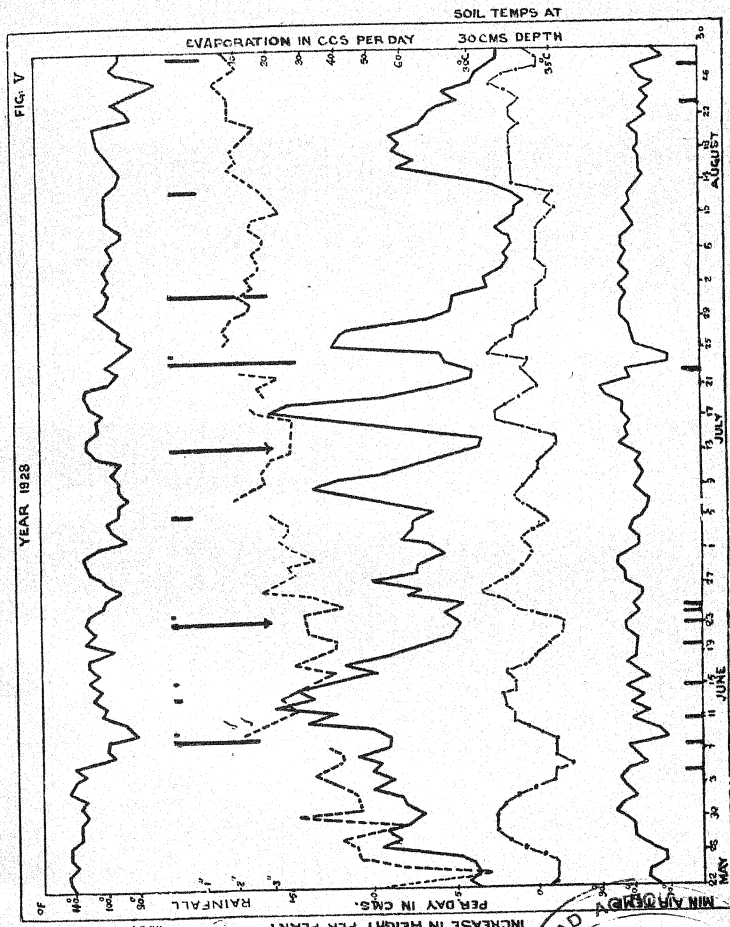


FIG. VI.

COMPARISON OF THE INCREASE IN HEIGHT OF THE MAIN STEM

WITH THE INCREASE IN LENGTH OF MONOPODIA OF

4-F

SOLID LINE . . . . MAIN STEM INCREASE

BROKEN LINE . . . . MONOPODIAL INCREASE.

INCREASE IN HEIGHT OF MAIN STEM AND INCREASE IN LENGTH OF MONOPODIA PER PLANT PER DAY IN CMS.

JUNE JULY AUGUST

In each of the Figures Nos. 3, 4 and 5, curves are drawn for comparison with the increase in height curves, showing the daily maximum and minimum air shade temperatures, the evaporation as measured by Piche-Cantoni Atmometers and the maximum soil temperatures at 30 cms. depth. The incidence of duststorms,† the irrigations and rainfall are also indicated.

(1) The effect of irrigation and rainfall.

In examining the increase in height curves for the three years, the most striking feature is the extent to which they are deformed from what would be expected to be a normal curve of growth. The response to irrigation or rainfall is perhaps the most striking feature of the curves, but, even after an irrigation or a shower of rain, the elongation response is not necessarily immediate, nor is it uniform. For example, on July 6, 1928, 0·6 inches of rain was followed by a greater rise than followed an irrigation (supposed to be the equivalent of three acre-inches) on the 23rd June. An irrigation on July 14 and rain on July 24 were not immediately followed by a rise; there was a delay of one day before the rise took place. In 1926, a considerable drop took place after two days' rain on the 12th and 13th of July. It is clear from the curves that other factors than the actual presence of water in the soil influence the availability of water for increase in length. It is, however, equally clear that rain, or the application of irrigation water, exercise a profound influence on the plant's capability to increase in height. There are one or two anomalies:—as for example, the excessive decrease in 1926 recorded for the 9th July * on the 4th day after an irrigation; and when other environmental conditions appear at least as favourable as on previous days. In this particular instance, it so happens that on the 10th July the plot was cultivated by hand, breaking up the soil crust. The accumulation of CO_2 in the soil had reached by the 9th of July such a magnitude that it began to affect root absorption. The hoeing permitted of soil aeration with the immediate result that growth again proceeded at a rapid rate. This is an example of how impossible it is to separate, entirely, the effects of one environmental condition from the effects of other environmental conditions.

It is, however, possible, on occasion, to attribute a particular growth manifestation to a particular cause. In the 1927 curve, for example, growth increased rapidly after the rains on the 5th and 8th July, and was maintained at a high level until the 15th July, when it fell rapidly to the 23rd July. The maximum and minimum temperatures and evaporation during the period of fall give no indication of a limiting effect when compared with the conditions prevailing, say during the period of rapid growth which culminated on the 26th June. The soil temperatures at 30 cms. during the period were rising, reaching 38°C. on the 21st of July, but cannot explain *in toto* the decrease in growth, for, as will be noted later, the soil

* Recorded on the morning of 10th July.

† Days on which duststorms occur are marked by short upright lines at base of diagrams.

temperature records do not represent exactly the soil temperatures under the crop, and also, in other years, equally big decreases in growth occur when soil temperatures cannot be supposed to be a limiting factor.

It seems, however, that the cause of this particular drop in growth can legitimately be ascribed to the phenomenon known as "physiological drought". Balls (4) states that the Egyptian cotton plant suffers for a portion of each day from a condition of physiological drought. An examination of the growth curves for different years leads to the belief that in the Punjab, the American crop—apart from a daily bout of physiological drought—also suffers from this disability for days at a time and that the only time when this condition is not a limiting factor is for the few days immediately subsequent to rain or irrigation. To return to the particular instance discussed above, we have the following conditions. Two heavy showers of rain in quick succession, totalling approximately $4\frac{1}{2}''$, fell on an alluvial medium heavy soil. The effect of rain is to puddle the upper surface of the soil, a crust being formed on drying. The air temperatures are fairly high, with a medium humidity permitting at least an average transpiration. We thus have conditions which tend to induce physiological drought—*viz.*, a soil in which capillary movement is slow, and—after the original oxygen brought in with the rain had been exhausted—a lack of oxygen in the soil, (and an accumulation of CO_2) which retards the absorptive activities of the roots, together with atmospheric conditions which, if water were available, would allow a normal removal of moisture by the root hairs from the soil.

To these observations it must be added that the water table is out of reach of the plant; moreover below the alluvial layers of soil are strata of sand which assist the rapid draining of the soil water to the deep water table. Certain observations (at present unpublished) in 1928 on soil moisture show that even irrigations in excess of the established custom are, on occasion, insufficient to maintain the soil moisture at its presowing content in the upper layers of the soil. The evidence points strongly to a state of physiological drought being responsible for many of the growth abnormalities shown by the growth curves. This condition must certainly be highly detrimental to the crop's progress.

(2) The effect of evaporation.

The question of evaporation may be next considered. For the measurement of evaporation, atmometers of the Piche Cantoni type obtained from Dr. E. B. Livingston were placed between the rows of cotton with the evaporating disc at a height of $16\frac{1}{2}$ inches from the soil surface. It would be expected, therefore, that irrigations should have a considerable effect on the evaporation as recorded by these atmometers.

It will be seen that in the early stages of the cotton, before the plant has grown up, there is a marked effect, but that when the plants are full-grown rain and

irrigations have little effect on the evaporating power of what may be called "the crop's atmosphere."

Between maximum temperatures and the evaporation records there is a marked connection in the daily fluctuations, as would be expected. The maximum soil temperatures at 30 cms. and evaporation are also correlated to some extent and Table II shows the actual figures. The 1926 and 1927 correlation coefficients are significant statistically; whereas the 1928 coefficient is not significant. The monthly correlation coefficients do not show much uniformity, nor are they always significant. Comparisons were made of the monthly coefficients with total rainfall during the month, and with the number of rainy days during the month but did not show any definite relationships, though rainfall obviously must affect both evaporation and soil temperatures. The linking of soil temperature and evaporation by rainfall would thus be expected, though it would tend to be obscured by irrigations, which reduce soil temperatures, without greatly affecting evaporation.

TABLE II.

Correlation between maximum soil temperatures (30 cms. depth) and evaporation in cubic centimetres.

Months	1926		1927		1928	
	r.	S. E. of r.	r.	S. E. of r.	r.	S. E. of r.
May	..	..	+0·45	±0·14	+0·37	±0·28
June	..	..	+0·20	±0·18	+0·41	±0·15
July	+0·55	±0·15	+0·48	±0·14	+0·16	±0·18
August	+0·47	±0·15	+0·02	±0·18	+0·42	±0·15
September	+0·65	±0·11	+0·33	±0·16	+0·55	±0·13
May to Sept. taken together	+0·49 (excluding May and June).	±0·09	+0·61	±0·038	+0·18	±0·08

The correlation coefficients, however, between evaporation and increase in height are distinctly discrepant. They are given in Table III together with the correlation coefficients for maximum soil temperature at 30 cms. and increase in height. It seems evident that at different times the conditions are such that the one or the other of these two factors (evaporation and soil temperature) tends to become limiting. Thus in July 1926, August 1927, and July 1928 the increases in height depend more on soil temperatures than on evaporation, in August 1926, June and July 1927, evaporation is the more important factor, while in other months neither factor is limiting

The evaporation curves * for the three years show that with the growth of the crop, the evaporation within the crop diminishes as would be expected. During the latter part of the season, the evaporating power of the air within the crop is low, and fairly constant. The general trend of the evaporation curves is to decrease after the middle of June each year.

(3) The effect of dust-storms.

Dust-storms appear to have little effect on the evaporation; for example, there was no increased evaporation on account of dust-storms on the 19th and 20th July 1926, the 2nd August 1926; the 10th and 25th August 1927 and the 20th June 1928. Dust-storms are frequently followed by increased humidity as mentioned earlier, and in consequence a reduced evaporation. Thus it appears that in a well-grown crop, the "crop atmosphere" is maintained at a fairly uniform level of humidity. Dust-storms can have little effect on the transpiration from the leaves as they generally occur at night, when the stomata are closed, and, as shown by the graph, have little effect on the evaporating power of the crop atmosphere. Yet dust-storms are seen to produce a drop in growth almost every time they occur. It seems difficult to believe that high winds have no effect on the availability of water for increase in length. The explanation appears to be that, although during a dust-storm the stomata may be shut, transpiration still occurs, and it occurs at that part of the daily cycle of growth—namely the early part of the night—when growth would normally be proceeding rapidly.

Transpiration would also be likely to occur most rapidly from the new tissue just behind the growing point, where elongation is proceeding most rapidly and the cuticle is thin. The effect on growth would thus be appreciable, while, as a dust-storm is generally of short duration, its total effect on the recorded evaporation (which is a summation of the whole 24 hours' evaporation) would be less noticeable, and might be entirely obscured by the conditions prevailing during the day, when evaporation is at its maximum. Another factor which may enter into the question is that of the mechanical effect produced by high winds. Bailey and Templeton (1) have published an account of the effect of handling cotton plants, and the same effect has been observed on cotton plants in the Punjab on which daily records of flowering, etc., have been taken. There is a marked stunting of growth. It is also a matter of common knowledge that trees growing in wind-swept situations bend before the prevailing wind, and are generally dwarfed or stunted.

The stunting of cotton due to handling, the drop in growth following dust-storms and the dwarfing of trees exposed to regular winds can probably all be ascribed to the same cause. The effect of handling cotton daily, during the morning, when the stomata are widely opened would be to increase transpiration slightly, and the accumulated effect of less water available for elongation would show at the end

*Note.—The evaporation and soil temperature curves for each year are inverted.

of the season, just as, during a duststorm, the effect of violent motion for a more prolonged period is reflected in the growth curve the next morning. It was actually found in 1927 that the average daily increase of plants measured weekly was greater in seven comparisons out of eight than the average daily increase of plants measured daily.

(4) The effect of soil temperatures.

In considering the effect of soil temperatures on growth, it must be noted that the soil temperatures were measured in an *open* patch in the centre of a cotton field. In 1928 when soil temperatures were taken in a plot of cotton, there was found to be a difference of approximately 3°C between the temperatures in the open, and those under cotton, at a depth of 30 cms. up to the end of July. From the beginning of August the difference widened owing to a fall in the temperatures under cotton. The increased shading effect of the plant seems the obvious explanation of this deviation.

There appears to be an obvious negative correlation between maximum soil temperatures and increase in height. In 1928 this apparent correlation though small is continued beyond the point where the soil temperatures under the crop deviate from the soil temperatures recorded in the diagram.

Table III.

Year	Month	Correlation coefficients of daily increase in height with—	
		Daily maximum soil temperature at 30 cms. depth	Daily evaporation through atmometers
1926	June	$r = +\cdot055 \pm\cdot21$	....
	July	$r = -\cdot633 \pm\cdot11$	$r = -\cdot056 \pm\cdot21$
	August	$r = -\cdot412 \pm\cdot15$	$r = -\cdot747 \pm\cdot087$
1927	June	$r = +\cdot021 \pm\cdot17$	$r = -\cdot653 \pm\cdot105$
	July	$r = -\cdot468 \pm\cdot14$	$r = -\cdot73 \pm\cdot087$
	August	$r = -\cdot764 \pm\cdot076$	$r = -\cdot255 \pm\cdot17$
1928	June	$r = -\cdot148 \pm\cdot18$	$r = -\cdot138 \pm\cdot18$
	July	$r = -\cdot677 \pm\cdot097$	$r = -\cdot053 \pm\cdot19$
	August	$r = -\cdot409 \pm\cdot15$	$r = -\cdot377 \pm\cdot15$

There may thus be little effect on the plant actually caused by soil temperature directly but the correlation coefficients in Table III between soil temperature and increase in height are sufficiently large to show that those environmental factors which cause high temperatures in the soil react unfavourably on the plant reducing its growth.

Camp and Walker (5) in pot experiments show that at soil temperatures of over 34°C the growth of cotton seedlings is decreased. In 1928 the recorded temperatures at 30 cms. never exceeded 36°C, and under the crop would be still less. Camp and Walker consider, however, that there may have been some other limiting factor operating which obscured the effect of soil temperatures on growth. Work has also been done by Cannon (6 and 7) on the growth of cotton roots at different temperatures and with different oxygen concentrations. These experiments were also on seedling plants. Further work is required on the relation of soil temperatures to crop growth in the field. The factors enumerated by Keen and Russell (8) as affecting soil temperature would require to be investigated individually.

(5) The effect of maximum and minimum air temperatures.

Finally, the maximum and minimum air temperatures remain to be considered. The correlation coefficients between daily increases in height and maximum and minimum air temperatures given in Table IV are not statistically significant and show that any direct temperature effect on growth can only be slight. In so far as air temperatures affect evaporation and soil temperatures, and are themselves affected by rain and cloud—which also affect evaporation and soil temperatures—

Table IV.

Correlation between daily increase in height of 4F Punjab-American cotton plant and maximum and minimum air temperatures.

—		1926	1927	1928
June	Max.	−0·44 ±·18	+0·05 ±·17	−0·20 ±·48
	Min.	+0·005 ±·22	+0·58 ±·11	+0·14 ±·15
July	Max.	−0·09 ±·18	−0·03 ±·18	−0·17 ±·55
	Min.	−0·12 ±·18	−0·10 ±·18	+0·07 ±·17
August and September	Max.	−0·37 ±·13	−0·28 ±·15	+0·34 ±·44
	Min.	−0·06 ±·16	+0·32 ±·15	+0·10 ±·16

they would exercise some small effect on growth, but it is obvious that under irrigated conditions, air temperatures would not necessarily affect the availability of water to the plant. But in view of Balls' work (3) this lack of direct effect with temperature is unexpected. Yet in each year numerous instances occur where growth increases in spite of high maxima, or decreases in spite of high minima, and *vice versâ*. A striking example is seen in 1926, where from the 2nd to the 9th July growth increases rapidly, even though the maximum air temperatures throughout are well above Balls' thermotoxic point. The watering on the 5th July accounts for this rapid growth, and shows that, provided water is available, air temperatures up to 116°F do not depress the growth of Punjab-American 4F. This is a striking testimony to the hardiness of 4F.

Summary.

The climate of the Punjab is generally reviewed during the cotton growing season and compared with that of some other cotton growing countries.

Diagrams showing the daily march of maximum and minimum air temperatures, evaporation in the cotton field, soil temperatures and the incidence of duststorms, rainfall and irrigation during the season are presented in comparison with the daily increase in height of Punjab-American Cotton 4F. These diagrams are briefly discussed. There is an increase in the rate of elongation following rain and irrigation and a temporary decrease following duststorms. A reason for the decrease following duststorms is suggested.

The evaporation in the "crop's atmosphere" decreases as the crop grows and becomes fairly constant. There appears to be a connection between maximum soil temperatures at 30 cm. depth in irrigated fallow and elongation.

The effects of maximum and minimum air temperatures on increase of stem elongation are found to be surprisingly small under irrigated conditions with 4F Punjab-American cotton.

Acknowledgment. The experimental work on Indian Cottons described in the paper was carried out by the Cotton Research section at Lyallpur which is financed partly by the Indian Central Cotton Committee and partly by the Punjab Government.

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